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THE SUGAR CONTENT OF WHEAT LEAVES INFECTED WITH STEM RUST

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SUMMARY

Two wheat varieties that differed in resistance to stem rust were grown at 65° and 85°F. Two weeks after inoculation with *Puccinia graminis* var. *tritici* race 15B, extracts of rusted and nonrusted seedling leaves were prepared and sugar determinations were made. Total sugar content was generally higher in leaves grown at 65°F than in leaves grown at 85°F, and was slightly higher in the variety Kenya 122 than in corresponding samples of Newthatch. Sucrose was the major fraction of soluble sugars present, and glucose was generally in somewhat higher concentration than fructose. In Newthatch the concentrations of the monosaccharides were not affected by temperature, but in Kenya 122 concentrations were higher at 85°F than at 65°F. Total sugar content, and especially the sucrose fraction, decreased with infection by stem rust, slightly in a resistant host and greatly in a susceptible one. The decrease in sugar was closely related to severity and kind of rust infection.

Earlier work with detached leaves indicated that carbohydrate supply must be adequate for rust infection (8). Later workers analyzed wheat to see if sugar content is correlated with resistance or susceptibility to stem rust. Daly (3) analyzed wheat leaves for several constituents, including sugars, and concluded that none of the fractions could be linked with resistance or susceptibility. Forsyth and Samborski (4) altered the resistance of several host varieties by treatment with DDT and maleic hydrazide and by searing the base of the leaves. These treatments, which made the wheat more susceptible to rust, increased the level of soluble carbohydrates in the leaves. Temperature treatments that altered the resistance of McMurachy wheat to race 15B of stem rust did not change the concentration of soluble carbohydrates. Lyles et al (7) analyzed isogenic lines of wheat that differed in their response to race 15B of stem rust, and wheat sprayed with maleic hydrazide. They concluded that a high level of soluble carbohydrate was strongly associated with resistance. Analyses of rusted material have shown that rust infection reduces the concentration of soluble sugars, but such analyses were often made on portions of adult plants inoculated earlier with the fungus (2, 10). In other instances, analyses of rusted leaves did not include analyses for the individual soluble sugars (11). The present experiments were made to determine the effect of infection by stem rust (*Puccinia graminis* Pers. var. *tritici* Eriks. & E. Henn.) on the content of the individual soluble sugars of wheat seedlings and the effect of temperature on the sugar content of these leaves.

Materials and methods.—An isolate of race 15B of *Puccinia graminis* var. *tritici*, obtained through the courtesy of the Cooperative Rust Laboratory in St. Paul, Minnesota, was used, and the inoculum was increased on Little Club wheat. Two wheat varieties were used: Newthatch C.I. 12318, from the Minnesota Agricultural Experiment Station, was susceptible to the rust isolate; Kenya 122 D.I.T. (L) C.I. 12186, from the United States Department of Agriculture, had a reaction sensitive to temperature, being resistant at 65°F but susceptible at 85°F. Seed were planted in an autoclaved 2:1 mixture of loam and sand in wooden flats (1.5 × 1.5 ft), and seedlings were inoculated and rust reactions were determined according to Stakman et al (13). All experiments were made in January, February, and March in adjacent greenhouses, one at about 65°F and the other at about 85°F.

Samples for sugar analyses were harvested from rusted and from nonrusted seedlings 13–15 days after inoculation of each variety grown at each temperature. First and second seedling leaves (uniformly infected in the rusted samples) were harvested in late afternoon, weighed rapidly, killed, and extracted with 80% ethanol according to the A.O.A.C. method (9). The alcohol-soluble fraction was concentrated to a few ml under reduced pressure at 40–45°C. Water-soluble material was transferred quantitatively with washings and made to a final volume so that 1 ml extract corresponded to 1 g fresh weight.

Sugars were determined according to a slight modification of the chromatographic method of Koch et al (6). On a sheet of Whatman No. 1 filter paper (7.5 × 22 in.) a starting line was drawn 4 in. from one end, and 2.25-in. margins were drawn on the long sides. In the 3-in. central section, 150 μl of extract were added in a band about 0.5 cm wide. Spots of the extracts and spots of a mixture of known sugars were placed in the marginal strips beside the central band. Chromatograms were irrigated 60–72 hours at 20°C with the organic phase of a butanol-ethanol-water (4:1:5 v/v) mixture. Side strips were removed following irrigation, and positions of the sugars were determined with ammoniacal silver nitrate. Strips corresponding to the known sugar positions were then cut from the central band and eluted with 25 ml water, and the eluate was filtered through coarse-porosity sintered-glass crucibles. In most cases glucose and fructose determinations were made on 1-ml aliquots of the eluate. For sucrose determinations, particularly in nonrusted samples, a

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further dilution (final volume 100–150 ml) was necessary.

Optical density readings following color development (1 ml eluate + 67 μl 80% phenol + 2.5 ml concentrated H_2SO_4) were made with a Coleman Junior spectrophotometer at 490 mμ, after using eluates of strips without sugars to adjust to 0 optical density. Standard curves of known chromatographed sugars in the range 10–40 μg were prepared, and sugar concentration was calculated from these curves and the known dilution factors. All determinations were in triplicate.

Results and discussion.—Severity of stem rust infection varied in the two experiments. Poor rust infection on Kenya 122 in experiment 1 prevented sampling rusted leaves, but rusted Newthatch leaves selected for analyses had 10–25 uredia on each leaf. In experiment 2, uredia were more numerous on both varieties of wheat. Race 15B produced infection type 4+[c] on Newthatch at 65°F in experiment 1, and infection type 4[cn] in experiment 2. Newthatch was also susceptible to 15B at 85°F, the rust infection type being 4++ in the first experiment and 4,4+ in the second. The reaction of Kenya 122 to race 15B was temperature-sensitive: rust infection was type 0:1 at 65° and type 4,4+ at 85°F.

Table 1.—Sugar content of seedling leaves of Newthatch and Kenya 122 wheats grown at 65° and 85°F (average of 3 determinations).

	mg sugar/g fresh weight							
	65°F				85°F			
	Newthatch		Kenya 122		Newthatch		Kenya 122	
	H[a]	R	H	R	H	R	H	R
Experiment 1[b]								
Fructose	0.7	1.4	1.0		0.7	1.3	2.1	
Glucose	1.0	0.8	1.4		1.0	1.6	1.9	
Sucrose	13.6	8.7	15.1		13.0	7.9	12.5	
Total	15.3	10.9	17.5		14.7	10.8	16.5	
Experiment 2[c]								
Fructose	0.6	0.3	0.6	1.0	0.5	0.4	1.0	0.5
Glucose	1.4	0.7	1.8	2.4	1.4	1.4	2.4	1.3
Sucrose	11.7	4.5	15.6	13.2	6.2	2.3	13.2	2.4
Total	13.7	5.5	18.0	16.6	8.1	4.1	16.6	4.2

[a] H denotes healthy nonrusted leaves; R denotes leaves rusted by wheat stem rust race 15B.
[b] Seed planted Jan. 12; seedlings inoculated Jan. 30 and Feb. 2; rust and sugars determined Feb. 15.
[c] Seed planted Feb. 18; seedlings inoculated Feb. 28; rust and sugars determined Mar. 14.

Sucrose, glucose, and fructose were identified in wheat leaf extracts, the quantitative data being given in Table 1. Sucrose was by far the major sugar. Several additional spots developed when chromatograms of leaf extracts were sprayed with ammoniacal silver nitrate. The R_F values of these spots indicated that these materials must be di- or tri-saccharides if they were true sugars. With more specific sugar sprays (1) it was concluded that these unknown spots were due not to sugars but to unknown reducing materials that react with the relatively nonspecific ammoniacal silver nitrate. Jain and Pelletier (5) recently detected several other sugars in rusted and nonrusted wheat leaves by the use of radioactive carbon dioxide. These compounds apparently were not present in sufficient quantity to be detected by the methods used in this study.

Rust infection of a susceptible host greatly decreased sugar content, mostly the sucrose fraction. The reduction may be due to increased respiration of the infected leaves (11). Later research showed that the respiratory pathway is different in infected leaves than that in healthy leaves (12). A slight reduction in total sugars, again primarily sucrose, occurred with rust infection of a resistant wheat (Kenya 122 grown at 65°F). The variation in sugar concentration with the severity and kind of rust development parallels Murphy's report (10) that the percentage of crown rust infection of oats is closely related to the reduction in sugar content. Even so, sucrose was still the major soluble sugar in rusted wheat leaves. In no case were the sugars completely exhausted even though the rust had sporulated.

Differences in sugar content between varieties and differences in sugar content due to the temperature at which the plants were grown were slight. Total sugar content of healthy nonrusted leaves was somewhat higher in Kenya 122 than in Newthatch. Both varieties, however, consistently had more sugars when grown at 65° than when grown at 85°F. In Newthatch, the simple monosaccharides (glucose and fructose) apparently were unaffected by temperature; in Kenya 122, in contrast, values were slightly higher in leaves at 85° than in leaves at 65°F. While these determinations were made 2 weeks after inoculation, the slight differences between varieties grown at the two temperatures do not indicate the total sugar content or the content of any one of the sugars was the factor determining rust reaction.—Department of Plant Pathology and Botany, University of Minnesota, Institute of Agriculture, St. Paul.

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THE FUNGICIDAL ACTIVITY OF ACROLEIN PHENYLHYDRAZONE AND RELATED COMPOUNDS AGAINST RUST

G. D. Thorn

Chemist.

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The author thanks the Plant Pathology section of the Institute, under R. A. Ludwig and more recently F. R. Forsyth, for the biological assays. Microanalyses were made by Dorle Bongart, of this laboratory.

SUMMARY

The candidate rust fungicide, Monsanto CP 8621, owes its fungistatic activity to acrolein phenylhydrazone and not to 1-phenyl-Δ^2-pyrazoline, the material one obtains on attempts to isolate the phenylhydrazone. *In vitro* and greenhouse tests with *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* and *Puccinia coronata* f. sp. *avenae* indicate that where it is possible to compare pairs of alkyl-substituted acrolein phenylhydrazones and the corresponding pyrazolines, the hydrazone is the more active as a rust suppressant.

The introduction of acrolein phenylhydrazone (I) as a candidate rust fungicide (Monsanta CP 8621) aroused my interest since this material has not been described in the chemical literature. Fischer and Knoevenagel (3) and von Auwers and Kreude (1) have shown that attempts to isolate the phenylhydrazone resulted in isolation of the product of internal cyclization, 1-phenyl-Δ^2-pyrazoline (II):

$$CH_2{=}CH{-}CHO + C_6H_5NHNH_2 \longrightarrow$$

$$[CH_2{=}CH{-}CH{=}N{-}NHC_6H_5] \longrightarrow \underset{\text{II}}{\begin{array}{l} N{-}N{-}C_6H_5 \\ \| \quad | \\ HC \quad CH_2 \\ \diagdown\!\diagup \\ CH_2 \end{array}}$$

I

Von Auwers and Kreude (1) were able to demonstrate the presence of acrolein phenylhydrazone in their crude reaction mixture, freed of phenylhydrazine, in 2 ways. Reduction with sodium amalgam yielded large amounts of aniline, and hydrolysis with dilute sulfuric acid gave phenylhydrazine. Neither reaction was shown by phenylpyrazoline itself. Fractional distillation of the crude reaction mixture, at reduced pressure, gave only phenylpyrazoline (25% yield), aniline, and a tarry residue. Investigations in this laboratory have confirmed the observations of von Auwers and Kreude in all respects.

The problem therefore resolved itself into whether the fungitoxic material obtained by the reaction of phenylhydrazine and acrolein is the isolable phenylpyrazoline, or whether it is indeed the acrolein phenylhydrazone, as CP 8621 is labeled. This was approached by a direct comparison of 1-phenyl-Δ^2-pyrazoline and the crude reaction mixture, and by use of those alkyl-substituted acroleins reported (1, 2) to give isolable phenylhydrazones as well as the substituted phenylpyrazolines.

Materials and methods.—The phenylhydrazones and pyrazolines were prepared according to methods reported in the literature: 3-methyl-1-phenyl-Δ^2-pyrazoline (2, 5); α-methylacrolein phenylhydrazone; (Shriner and Sharp (6) considered their product to be 4-methyl-1-phenyl-Δ^2-pyrazoline, but spectroscopic evidence (2) indicated that they had indeed the hydrazone); 4-methyl-1-phenyl-Δ^2-pyrazoline (2); crotonaldehyde phenylhydrazone (1); 5-methyl-1-phenyl-Δ^2-pyrazoline (2); α-ethylacrolein phenylhydrazone, m.p. 50–52°C. Anal: N, 15.95%. Calc. for $C_{11}H_{14}N_2$: N, 16.08%; 4-ethyl-1-phenyl-Δ^2-pyrazoline, $b_{0.07}$ 87°C. Anal: N, 16.16%. Calc. for $C_{11}H_{14}N_2$: N, 16.08%; 2-methyl-2-pentenal phenylhydrazone (1); 4-methyl-5-ethyl-1-phenyl-Δ^2-pyrazoline (1); 3,5,5-trimethyl-1-phenyl-Δ^2-pyrazoline (1).

The "crude reaction mixture" from acrolein and phenylhydrazine was obtained by reacting the components in either solution at 0°C, extracting exhaustively with cold 2% sulfuric acid to remove unreacted phenylhydrazine, and evaporating the ether layer to dryness at room temperature in a rotary evaporator.

Biological activity was measured by spore-drop assay, using *Monilinia fructicola* (Wint.) Honey as the test organism, against wheat leaf rust (*Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* and oat crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Eriks. & E. Henn.), both *in vitro*, by the method described by Forsyth and Unwin (4), and as pre- and postinfection spray of 7-day-old seedlings, and against cucumber mildew (*Erysiphe cichoracearum* DC.).

The wheat and oat seedlings were inoculated by applying rust uredospores in suspension with a commercial atomizer (paint spray gun) to the seedlings, 8–10 per pot, on a turntable. The suspension of spores

was prepared by shaking 100 ml distilled water containing 3 drops of sulfonated castor oil with about 50 mg of rust spores. This suspension was used to inoculate 40 pots of seedlings. For postinfection tests the chemicals were applied by atomizer spray 5 days after inoculation; in the preinfection tests the plants were inoculated immediately after the spray deposit of test chemical had dried on the leaf.

Results.—On physicochemical grounds, the (substituted) acrolein phenylhydrazones can be differentiated from the corresponding 1-phenyl-Δ^2-pyrazolines on the basis of characteristic differences in ultraviolet absorption spectra. All Δ^2-alkenyl phenylhydrazones examined show strong absorption bands at approximately 325, 300, and 247 mμ, whereas the 1-phenyl-Δ^2-pyrazolines display absorption maxima at about 280 and 245 mμ (Fig. 1). The "crude reaction mixture" from acrolein and phenylhydrazine shows absorption maxima at 321, 290, and 245 mμ, which strongly supports the chemical evidence, mentioned above, for the existence of acrolein phenylhydrazone. As earlier workers had found, it was not possible to isolate the hydrazone by distillation at reduced pressures. Nor was partitioning by paper chromatography successful.

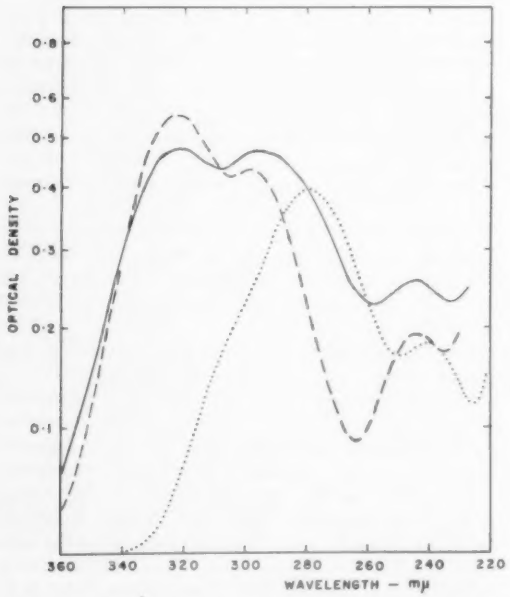

Fig. 1.—Ultraviolet absorption spectra in ethanol solutions of the "crude reaction mixture" of phenylhydrazine and acrolein (——), 1-phenylpyrazoline (. . . .), and α-methylacrolein phenylhydrazone (— — —).

Although acrolein phenylhydrazone cyclizes very readily to the phenylpyrazoline, it might be noted that certain alkyl-substituted acroleins form much more

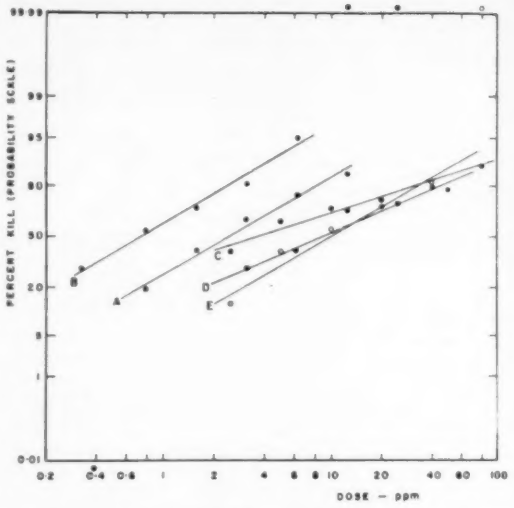

Fig. 2.—*In vitro* comparison of fungicidal activity against *Monilinia fructiocla* of **A)** "crude reaction mixture" of phenylhydrazine and acrolein; **B)** 5-methyl-1-phenylpyrazoline; **C)** 2-methyl-2-pentenal phenylhydrazone; **D)** α-methylacrolein phenylhydrazone; and **E)** 3,5,5-trimethyl-1-phenylpyrazoline.

stable phenylhydrazones. For example, α-ethylacrolein phenylhydrazone is isolable by fractional distillation along with 4-ethyl-1-phenylpyrazoline, having been formed under reaction conditions that are normally used to ensure cyclization (heating under reflux the aldehyde and phenylhydrazine in aqueous 3% NaOH).

Table 1 presents in terms of approximate ED_{50} values the biological activity of some of the various phenylhydrazones and phenylpyrazolines as measured in the spore-drop assay with *Monilinia fructicola* as the test organism. The ED_{50} values for the phenyl hydrazones that could be isolated are on the same order as those of the corresponding phenylpyrazolines. A significant difference is noted, however, between the activities of pure 1-phenylpyrazoline and of the "crude reaction mixture." Fig. 2 presents the dosage response data for several of the compounds tested. Within the limits of the data, parallel slopes appear to be given by the "crude reaction mixture," the hydrazones, and the pyrazolines for which the data are shown, implying the same mechanism of fungitoxic action towards *M. fructicola* by all these chemicals.

The *in vitro* activities of acrolein and crotonaldehyde phenylhydrazones, and the corresponding pyrazolines, against the organisms causing wheat leaf and crown rusts are given in Table 2. Two observations can be drawn from these data. Wheat leaf rust is more susceptible than oat crown rust to each of these chemicals, and in each case the hydrazone is more fungitoxic than the corresponding pyrazoline. Preinfection spray tests in the greenhouse with the same organisms and chemicals permit the same conclusions.

Table 1.—ED_{50} values (ppm) for alkyl-substituted acrolein phenylhydrazones and 1-phenylpyrazolines as obtained from spore drop assay, *Monilinia fructicola* as test organism.

Carbonyl compound	ED_{50} of phenylhydrazone	Phenylpyrazoline	ED_{50} of pyrazoline
acrolein	2[a]	no substituents	>100
3-butenone-2	–	3-methyl-	>100
α-methylacrolein	4	4-methyl-	3
crotonaldehyde	~1	5-methyl-	0.7
2-methyl-2-pentenal	3	4-methyl-5-ethyl-	2
mesityl oxide	–	3,5,5-trimethyl-	8

[a] Crude reaction mixture.

Table 2.—*In vitro* toxicity (% kill) of acrolein and crotonaldehyde phenylhydrazones and the corresponding phenylpyrazolines to wheat leaf and oat crown rust organisms.

Compound	Organism	Concentration (ppm)						
		100	50	25	12.5	6.25	3.13	1.56
Acrolein phenylhydrazone[a]	Leaf rust			92	67	40	11	0
	Crown rust			29	14	0	0	0
1-phenylpyrazoline	Leaf rust	100	93	26	0	0		
	Crown rust	97	37	14	0	0		
Crotonaldehyde phenylhydrazone	Leaf rust				64	50	0	0
	Crown rust				39	14	0	0
5-methyl-1-phenyl-pyrazoline	Leaf rust			18	10	20	0	0
	Crown rust			9	10	9	0	0

[a] Crude reaction mixture.

Table 3.—Postinoculation control of oat crown rust by various pyrazolines and hydrazones.

Chemical	Spray strength (ppm)				
	500	250	125	62	31
3-methyl-1-phenylpyrazoline	0[a]	0	0	0	0
crotonaldehyde phenylhydrazone	75	50	0	0	0
5-methyl-1-phenylpyrazoline	35	10	0	0	0
2-methyl-2-pentenal phenylhydrazone	100	90	35	0	0
4-methyl-5-ethyl-1-phenylpyrazoline	90	75	0	0	0
α-methylacrolein phenylhydrazone	100	90	75	25	0
4-ethyl-1-phenylpyrazoline	0	0	0	0	0
3,5,5-trimethyl-1-phenylpyrazoline	25	0	0	0	0

[a] Values are expressions of arrest of rust development, 100 signifying complete (100%) control.

CP 8621 (technical acrolein phenylhydrazone) gave a measure of control of wheat leaf rust also when applied as a postinfection spray. Control was complete at a spray strength of 2500 ppm; some pustule development was observed with 625 ppm. Under the same conditions, pure phenylpyrazoline gave no control, even at 5000 ppm.

Experimental results on postinfection control of oat crown rust by several of the hydrazones and pyrazolines are presented in Table 3. As with wheat leaf rust, a measure of control was obtained with the hydrazones. The corresponding pyrazolines were less effective. 3-methyl- and 4-methyl-1-phenylpyrazoline were ineffective at 500 ppm, the top level tested. It should be noted, however, that in all cases showing control, only a suppressive action was actually attained. The data of Table 3 were obtained 2 days after application of the chemical. If the test plants were observed after 7 days, however, little or no control was in evidence. Thus, these chemicals merely delay development of the rust lesions.

None of the hydrazones and pyrazolines gave any control of cucumber mildew in greenhouse tests.

As might be expected, the results of spore-drop assays with *M. fructicola* do not properly assess the behavior of the substituted acrolein phenylhydrazones and phenylpyrazolines toward wheat leaf and oat crown rusts on the plant. The *in vitro* assay technique with the rust organisms, however, gave toxicity data that parallel the results of greenhouse tests and therefore could be used as a simple quick method for screening the activity of chemicals against these organisms.

In the case of acrolein phenylhydrazone (either as CP 8621 or the "crude reaction mixture") vs. phenylpyrazoline, the former is active against the two rusts,

but only as a suppressant, whereas the latter compound appears quite inactive. Where it has been possible to compare pairs of substituted hydrazones and pyrazolines, the hydrazone is more active as a rust suppressant.—Pesticide Research Institute, London, Canada.

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A LEAF BLIGHT OF CIMARRON OATS

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SUMMARY

A leaf blight disease, herein named Cimarron blight, was found to be peculiar to the oat variety Cimarron and its genetic derivatives. Neither of 2 fungal organisms consistently isolated from diseased tissue was found to be the causal agent. Comparative histological studies of diseased leaf tissue showed that the appearance of lesions was associated with a collapse of mesophyll tissue. There was no difference in disease expression in Cimarron oats grown from chemically or hot-water-treated seed and those grown from non-treated seed. Cimarron oat plants grown under sterile conditions developed disease lesions. The reactions of F_2 hybrids indicated that susceptibility to the disease is recessive. Several relatively stable selections from Cimarron × Traveler varied considerably in susceptibility. The results suggest that Cimarron blight is not parasitic, but a genetically controlled physiological breakdown of leaf tissue, with expression influenced by environment.

The oat variety Cimarron C.I. 5106 was released by the Oklahoma Agricultural Experiment Station in 1954. Schlehuber (6) described its origin and performance in detail. The variety was not recommended for eastern Oklahoma, largely because of its susceptibility to rust and to an unidentified leaf disease known locally as "Cimarron blight." This disease is known to occur only on Cimarron oats or its genetic derivatives, often reducing the effective leaf surface by more than 65%.

H. R. Rosen (personal correspondence, to H. C. Young, Jr., 1953) reported from Arkansas the production of blight symptoms following inoculation of Cimarron oats with a spore suspension of *Cladosporium* sp. Numerous isolations at Oklahoma State University prior to the present work, however, failed to yield any organism with sufficient consistency to be regarded as a causal agent.

A heritable blotch of a similar nature, not so restricted to genotype, has been described on oats by Ferdinandsen and Winge (2). A manganese deficiency disease of oats resembling Cimarron blight and expressed as a leaf spot (gray speck) has been described by Samuel and Piper (5). Chester (1) discussed the cause of "physiological leaf spot" of oats and other cereals.

Symptomatology.—The disease symptoms are first recognizable when small chlorotic spots begin to appear on the leaf blade, more often on the proximal half (Fig. 1-A,B). The spots are usually near the midrib but may begin at the margins. They soon develop into large oval to long irregular necrotic areas that become buff to light-brown. The lesions may have a sharply defined margin. Some of the larger blighted areas show a concentric pattern (Fig. 1-A,B,C,D). This is more evident on the broad coarse leaves of plants growing in the field. As the spots increase in size, necrosis often advances more rapidly along the midrib (Fig. 1-E).

During the later stages of disease development, spots usually coalesce and extend the entire length and width of the leaf (Fig. 1-F,G). The blight advances rapidly at this stage. The proximal portion of the leaf blade may appear completely dead while the distal portion remains green for some time. Death of the tissue gradually proceeds upward until the entire leaf becomes shriveled and brown.

The blight symptoms invariably occur first on the lower, more mature leaves, with successive leaves attacked as they approach maturity. In plants growing in the greenhouse, however, the upper leaves were sometimes more severely affected even though they were the last to show symptoms.

Occurrence.—Symptoms were never observed to occur on Cimarron oat seedlings before they had reached the age of 9 weeks. That was true of both plants grown in the greenhouse and plants grown in the field. Cimarron oats planted at 5-10-day intervals in the greenhouse from October to April, always exhibited symptoms between the 9th and 12th week after planting. In fact, at that time of year it was

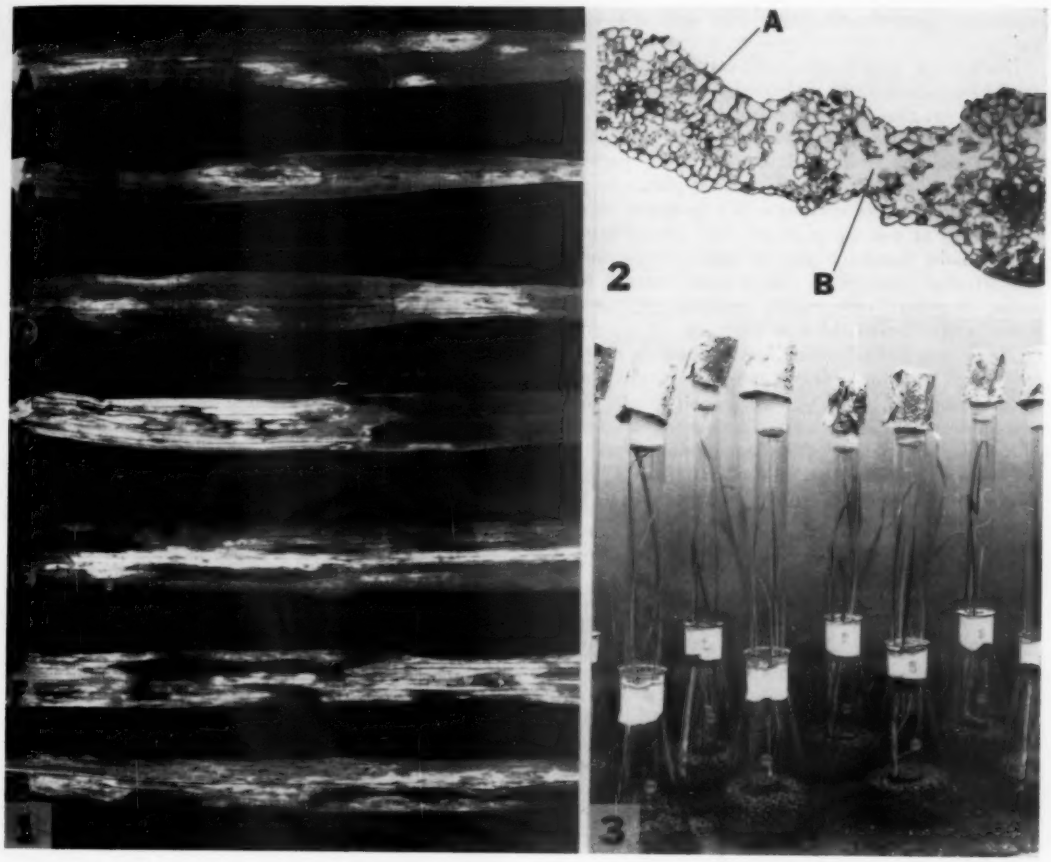

Fig. 1–3.—**1)** Leaves of Cimarron oats illustrating symptoms of the Cimarron blight. **A,B,C)** Lesions in the early stages of development; **D,E,F,G)** lesions in more advanced stages of development. **2)** Transection of Cimarron variety oat leaf with a lesion in the early stage of development. **A)** Region of normal tissue. **B)** Mesophyll tissue disintegration in an area of the lesion (× 125). **3)** Oats of the varieties Cimarron and Traveler growing in containers devised to provide an aseptic environment.

impossible to grow the variety in the greenhouse without symptoms on all plants.

In test plots containing several oat varieties, Cimarron is often conspicuous because of the large number of dead and blighted leaves at the base of the plants. Secondary invaders also apparently play some part in symptom expression, especially as the plant approaches maturity in the field.

Cimarron blight has been associated with this variety since its initial selection. The blight has been of little consequence in western and northwestern Oklahoma, but at times has been severe in the eastern portion of the state. This is probably due to climatic factors since the disease is more severe in some winters than others in a single locality.

Experimental results.—*Isolation experiments.*—Diseased tissue showing typical symptoms in all stages of development was collected from Cimarron plants grown in either greenhouse or field. Fungal and bacterial organisms were isolated from small leaf sections that had been washed and sterilized in one or a combination of the following solutions: 1) $HgCl_2$ (1:1000), 2) 70% ethanol, 3) 0.5% NaOCl. The isolation media were potato-dextrose agar, water agar, glucose-peptone agar, and oxgall agar.

More than 500 different lesions yielded 2 organisms the most frequently. Unidentified species of *Alternaria* and *Helminthosporium* appeared frequently on material from the field but less often in isolations from greenhouse plants. During subsequent identification of isolates, none resembled *Cladosporium*.

Several separate isolates of *Alternaria* and *Helminthosporium* were retained for use in inoculation experiments. The most commonly occurring bacteria were selected from each group of isolations and transferred to agar plants for comparison. Bacterial colonies appeared rather regularly throughout the experiments. They were considered to be contaminants or secondary

organisms, because no single type occurred consistently.

Inoculation experiments.—Three organisms were tested for pathogenicity: *Alternaria* sp. and *Helminthosporium* sp. that had been isolated previously, and cultures of *Cladosporium* obtained from H. R. Rosen. Glucose-peptone broth and oatmeal broth were used to increase the inocula. Cimarron oat plants grown in the greenhouse were sprayed with a spore and mycelial suspension at 6–8 weeks of age and placed in sheet-plastic moist chambers. For 10 days following inoculation, treated and control plants were returned to the moist chambers each night, sprayed with distilled water, and removed the next morning.

There was no difference in the reaction of plants inoculated with the different fungi. Typical Cimarron blight symptoms began to occur on all plants, including uninoculated controls, by 12 weeks after planting.

Histological studies.—Diseased and normal tissue for microscopic examination was secured from Cimarron oats grown in the greenhouse. The material was killed and fixed in a Craf III solution, using a dioxon dehydrating series. Serial sections of leaf, leaf sheath, and stem were stained with safranin and fast green.

There was no indication of bacteria or fungi in any of the lesions examined, nor was there evidence of mechanical injury. No hyperplasia or hypertrophy was apparent.

Blight lesions were first distinguishable in leaf transverse sections as areas where the mesophyll had begun to collapse (Fig. 2). These areas occurred most frequently adjacent to the vascular bundle of the midrib or near one of the major veins. Subsequently, cells of the bundle sheath and epidermis collapsed and tissues in the diseased area disintegrated, leaving only the sclerenchyma and xylem elements intact.

The section shown in Fig. 2 is typical of many examined. Disease expression was similar in both the leaf blade and sheath. Lesions were never found on stem tissue.

Seed-treatment experiment.—Since the disease potential seemed to be universally present, the possibility of a seed-borne incident was examined. Cimarron oat seeds were treated by 3 methods to determine the possible effects of seed treatment on disease development: 1) seed was treated with a 1% NaOCl solution under vacuum for 7 minutes, 2) seed was presoaked in water at room temperature for 14 hours, placed in water at 53°C for 13 minutes, and immediately immersed in cool water and dried, and 3) seed was treated with Arasan and Ceresan M at rates recommended by the manufacturer. Controls were planted.

The seed was placed in a germinator at 65°F for 48 hours before planting. Germination was reduced to 56% in the hot-water-treated seed. The germinated seed was then planted in 6-in. pots in the greenhouse and in 5-ft rows in the field. Plants were grown to maturity with no difference between treatments and controls. Symptoms developed on all plants in both greenhouse and fields.

Sterile-plant experiment.—Containers were devised to facilitate growing oat plants over an extended period under sterile conditions. The development of blight symptoms under such an environment would preclude the possibility of an air-borne pathogen as the causal agent. A modification of apparatus used by German and Bowen (3) and Kathrein (4) was developed. For this purpose a 2-L wide-mouth Erlenmeyer flask, partially filled with vermiculite, formed the basic component (Fig. 3). The whole container was thoroughly autoclaved twice. Surface-sterilized seeds were germinated on potato-dextrose agar in Petri dishes. The sterile germinated caryopses of Cimarron and the variety Traveler C.I. 4206 oats were added, aseptically, one to each of 8 containers. Five flasks contained Cimarron and 3 contained Traveler. A sterile complete nutrient solution was added aseptically to the containers as it was needed. These plants were grown from February through May, 1956.

Growth of the sterile plants was very rapid during the initial weeks. Leaves were a healthy green with no noticeable etiolation. At 9½ weeks, all 5 of the Cimmarron plants began to show foliar spotting resembling the early stages of Cimarron blight. Blighted areas developed along the midrib of the leaf blades and rapidly progressed longitudinally. Nothing of a similar nature developed on the control variety, Traveler.

Inheritance studies.—Seed produced by F_1 plants from the cross Cimarron × Traveler were grown to determine the heritability of Cimarron blight. The initial crosses were made in 1947, and the F_1 plants were grown in 1948. Thirty per cent of the seed from the Cimarron × Traveler crosses germinated. Respective germination of the Cimarron and Traveler parent seeds of a similar age was 0 and 4%. The F_2 seedlings were grown individually in 6-in. pots in the greenhouse. Later, some were moved to a cold frame. All individuals originating from a single F_1 plant, were given similar treatment, however. This study covered a period from January to July, 1956.

At 3 months, F_2 plants could be classified by 3 categories: resistant, intermediate, susceptible. Those in the intermediate group developed chlorotic spots and streaks indicative of the early stages of Cimarron blight, but failed to develop the more advanced symptoms. The disease reactions of 196 F_2 individuals are summarized in Table 1.

Observations were made on the Cimmarron blight reaction of several selected lines that had originated from crosses between Cimarron and Traveler. These lines were in the F_9 and F_{10} generation. All lines had been selected primarily for their Cimarron-type agronomic characteristics, and all were susceptible to Cimarron blight. On the basis of a disease rating ranging from 0 to 4, Cimarron averaged 2+, C.I. 7128 averaged 3, and Stw. 513795 averaged 1+. These observations are summarized in Table 2.

Discussion.—At the outset of these studies, the experimental procedure was based on the assumption

Table 1.—The Cimarron blight reaction of F_2 plants from the cross Cimarron × Traveler oats.

F_1 plant number	No. F_2 plants	No. resistant	No. intermediate	No. susceptible
48 Stw. 9708-1[a]	36	25	8[b]	3
48 Stw. 9708-2	84	43	33	8
48 Stw. 9709-2	12	8	3	1
48 Stw. 9709-3	64	33	18	13
Total	196	109	62	62

[a] Number assigned to plants by the Agronomy Dept., Okla. State University.

[b] Intermediate refers to individuals that had chlorotic spotting and lesions similar to those of Cimarron blight in the early stages, but failed to develop more severe symptoms.

that a fungus was the causal agent of the Cimarron blight disease. This was supported by two factors: 1) a fungal pathogen had apparently been isolated and identified as the causal agent; and 2) the disorder in the field had been observed to react like a fungus disease.

The results, however, indicate that neither a bacterium nor a fungus is involved as an incitant of the disease. The organisms isolated from diseased plant tissue appear to be secondary invaders and have a minor effect on symptom expression.

It has been observed that fall plantings of Cimarron oats in the field, are more severely affected than spring plantings. Similar observations have been made with plants grown in the greenhouse. Thus it appears that disease development is influenced by environment, both in greenhouse and field. Temperature, light intensity, and photoperiodicity appear to be the more important external factors in disease expression.

Expression of symptoms is apparently an inherited character since both resistant and susceptible types occurred in the progeny of crosses between Cimarron and Traveler oat varieties. Examination of F_2 plants indicated that more than one gene was involved and that dominance was incomplete, but the data failed to fit any known genetic ratio. These results may have been modified by 2 factors: 1) the environment could have influenced symptom expression in plants grown during this season of year; and 2) the low germination could have been a selective factor in altering the ratios obtained. Observations of several selections from Cimarron × Traveler crosses in later generations showed that different stable lines may possess a constant, but different, degree of susceptibility to the disease. One selection, C.I. 7128, consistently showed a more severe disease reaction than the other lines, including Cimarron.

Table 2.—The reaction to Cimarron blight of Cimarron and some Cimarron × Traveler selected lines.

Variety or line	Blight Reaction[a]			
	Disease Obsn. Nursery 1954[b]	Rust Nursery 1956[b]	Pure Seed Increase 1956[b]	Greenhouse 1956
Cimarron (C. I. 5106)	2+, 3	2–	2	2
Stw. 513705	3, 3+			1+
Stw. 513795	1, 1	1		0–1
Stw. 513861 (C. I. 6989)	2, 2	0–1	1	0–1+
Stw. 513714 (C. I. 7128)	3, 3+	2	2+	2+
Stw. 513694 (C. I. 6988)	1, 3	1+	1+	1

[a] The severity of reaction to Cimarron blight is based on a linear scale of 0–4, from absence of symptoms to disease in its most severe form (75% or more of the leaf surface destroyed).

[b] On the Agronomy Farm, Oklahoma State University, Stillwater.

Finally, these studies have indicated that the cause of Cimarron blight is not a parasitic agent but a heritable predisposition to a physiological tissue breakdown. Although expression of the disorder may be altered somewhat by the environment, symptoms invariably occur at a particular stage of physiologic maturity of the foliar tissue.—Department of Botany and Plant Pathology, Oklahoma State University.

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RELATION OF HISTOCHEMICAL TESTS FOR PHENOLS TO VERTICILLIUM WILT RESISTANCE OF POTATOES

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SUMMARY

The relative concentrations of *o*-dihydroxy phenols, chiefly chlorogenic acid, was estimated in 12 different tissues of 6 potato varieties from germination to maturity by the use of a 10% ferric chloride solution. Methyl red also disclosed the presence of chlorogenic acid as indicated by a lower pH value in the tissues.

Concentrations of phenolic compounds were extremely high in the vascular systems, the root tips, and the sprout tips of all young potato plants. The concentrations in the vascular tissues of the underground stem, stem node, stolon, tuber, and root stele were greater and more prolonged in 3 resistant varieties, Populair, 41956, and Great Scott, than in 3 susceptible varieties, Russet Burbank, Early Gem, and Kennebec.

As the plants developed and matured, phenolic concentrations decreased more rapidly and to a lower level in susceptible varieties than in resistant varieties. In the field, Verticillium wilt developed more rapidly and more severely in the susceptible varieties, coincident with or following the decrease of phenols in the vascular systems. When plant maturity was delayed by defoliation or by growth regulators, a decrease of phenolic concentrations in the plant was also delayed. A similar delay was found in the Verticillium wilt disease in the field.

The intensity of the ferric chloride test of the underground stem node had a highly significant correlation with resistance to Verticillium wilt, as did the ferric chloride reaction in the cortex of freshly harvested tubers. The cortical test was sufficiently accurate for rough screening of varieties or selections for resistance.

Ferric chloride reaction of the periderm of tubers produced a highly significant correlation with scab resistance.

Chlorogenic acid in distilled water inhibited spore germination and mycelial growth of *Verticillium albo-atrum*. The concentrations of *o*-dihydroxy phenols in young, actively growing potato plants, and in resistant plants were much greater than the inhibitory levels *in vitro*.

Introduction.—When numerous varieties and selections of potato (*Solanum tuberosum* L.) were tested for scab resistance by the ferric chloride method (2), a few lines gave a positive green-color reaction of the periderm of the tuber, even though these lines were not resistant to scab. Further examinations disclosed a reaction to ferric chloride in the cortex of the tuber of many of the lines. Comparisons with field records indicated that the strength of the cortical reaction was correlated with resistance to Verticillium wilt caused by *Verticillium albo-atrum* Reinke & Berth. It was also found that a ferric chloride test of the node of the underground stem of young plants indicated their relative resistance to Verticillium wilt.

A study was conducted to determine the effect on Verticillium wilt resistance of naturally occurring phenols in the underground portions of potato plants.

Materials and methods.—The content of chlorogenic acid and other phenols in various portions of the potato plants was determined both histochemically and by paper chromatography. Chromatographic methods were described by Lee and Le Tourneau (4).

Underground portions of potato plants were used for the histochemical tests. Chlorogenic acid was believed to be chiefly responsible for the green-color reaction obtained with ferric chloride (2, 4, 8). The various tests for chlorogenic acid described by Reeve (9), although adequate, did not appear to be superior to ferric chloride for tissue testing. Methyl red, of the common pH indicator dyes, gave a distinctive red color to the same tissues in which ferric chloride and other tests for *o*-dihydroxy phenols were effective.

Six varieties of potatoes were tested at about 10-day intervals from sprout development to maturity. Both ferric chloride and methyl red were applied to 12 different locations on the underground portions of the plants. A few drops of a 10% solution of ferric chloride, a specific test for *o*-dihydroxy phenols, were applied to a free-hand section in a watch glass. The tissue was then crushed with the edge of a glass slide. Duplicate sections were treated with a .05% methyl red solution without maceration. All readings were made against a white background with light from a fluorescent fixture containing four 40-W tubes.

Visual units were established for recording increasing intensity of color response: 0–6 for the green from ferric chloride, and 0–10 for red from methyl red. With young sprouts, an extension to 50 units was necessary to represent the higher intensities found with both reagents.

A glass hypodermic syringe and needle were used in testing tuber periderm for scab resistance (2), and the tuber cortex for *o*-dihydroxy phenols. A few drops of fresh ferric chloride solution were placed on the surface to be tested, and several scratches with the needle brought out the color reaction.

For determination of the effect of delayed maturity on the phenolic content, plants of each variety were defoliated 45 and 60 days after planting by stripping all leaflets from the midribs of the leaves. The stem pith of other plants 45 days old was injected with 0.1

ml of a 50-ppm solution of either methyl ester of naphthalene acetic acid or 2, 4, 6-trichlorobenzoic acid. Treated plants resumed growth after defoliation or injection, but matured later than untreated plants of the same varieties.

To determine the inhibitory effect of different concentrations of chlorogenic acid on the conidia of *V. albo-atrum*, chlorogenic acid was added to boiled distilled water at concentrations ranging from 0 to 10,000 ppm. The pH was adjusted to an acid limit of 5.0 in one series, but in the other series was allowed to become more acid with the increased addition of chlorogenic acid. Spores were taken from actively growing plate cultures by repeated washing with a few drops of the test solution in a medicine dropper. Duplicates of each test were placed in double, shallow culture slides. After the spores had settled, the solution was replaced with fresh solution at 24-hour intervals to keep the concentration constant. A few fragments of mycelium were inadvertently carried along with the spores. Several hundred spores of each treatment were counted under oil immersion after staining with methylene blue. Notes were also taken on the structural differences of the spores and mycelial strands at the various concentrations. The entire series was repeated, with 4 samples for each treatment.

Correlation coefficients were calculated to compare the various histochemical tests with field resistance to Verticillium wilt. Used for field readings was a Verticillium wilt index (6) that incorporated both amount and severity of disease. Index values of 10, 30, or 50 respectively indicated all plants slightly, moderately, or severely affected. An index of 100 indicated that all plants were dead. Analysis of variance was used to determine the significance of differences between reagents and locations of the histochemical tests.

Results.—*Chromatographic.*—The chromatographic determinations (4) showed that chlorogenic acid was present in roots, young tubers, sprouts, and foliage of the potato plants. In sprouts and tubers it appeared to be the major phenol present.

Other compounds besides chlorogenic acid, fluoresced or reacted with one or more of the spray reagents. Tyrosine and histidine were found in tubers, sprouts, and foliage, and tryptophane in sprout and tuber extracts. These compounds do not contain *o*-dihydroxy groups and would not interfere with histochemical tests for chlorogenic acid. Tubers stored for some time contained a material that corresponded to a known *o*-dihydroxy compound, caffeic acid, similarly to findings of Johnson and Schaal (3).

Other compounds observed on chromatograms have not been identified. Foliage extracts contained phenol pigments tentatively classified as anthocyanins. One anthocyanin was pink to light red on the unsprayed chromatogram and became blue to purple when sprayed with sodium carbonate. It fluoresced orange-red under ultraviolet light and had an Rf of 0.30–0.35 in butanol–acetic acid-water. Another material that gave a reaction with sprays and ultraviolet light was noted at Rf 0.15–0.20. An anthoxanthin-like material gave a light-yellow spot on the unsprayed chromatogram and became dark yellow-brown when sprayed with sodium carbonate. It exhibited a brown fluorescence under ultraviolet light, and an Rf of 0.45–0.50 in butanol-acetic acid-water.

Histochemical.—The ferric chloride test of the tuber periderm showed a highly significant correlation with scab resistance ($r = 0.678$) in over 400 varieties and selections. This agreed with the findings of Johnson and Schaal (2).

Ferric chloride tests of the tuber cortex showed a highly significant correlation ($r = 0.376$) with field resistance to Verticillium wilt in 200 lines of potatoes. Reactions of the underground stem nodes of 100 lines to ferric chloride also showed a highly significant correlation ($r = 0.444$) with resistance to Verticillium wilt.

Positive reactions to both ferric chloride and methyl red were found in the vascular system of the stems, stolons, and tubers of young plants, in the root stele, at underground stem nodes, and in the periderm and lenticels of the tubers. The cortex of the roots, and the cortex and the pith of the stems, stolons, and young tubers, generally gave no color reaction. The pith and cortex of young tubers of the Populair variety, however, gave a color reaction. High concentrations of phenols were observed in the root-tips, in young sprouts, and at the point of juncture of the root and stem vascular systems. Concentrations were extremely high in sprout tips and root primordia.

Tubers of varieties that had given strong reactions in the vascular system, cortex, and pith within 30 days of harvest showed a reduced concentration after storage for a few months. When the tubers had started to sprout, only a faint reaction could be found in the vascular system of the tuber, none in the cortex or pith, and only slight in the periderm in many cases. A similar decrease in concentration in the periderm was found by Johnson and Schaal (3).

The extremely high concentrations of phenols in the vascular tissues and the tips of the sprouts, together with a declining concentration in other portions of the tuber, suggested that the phenolic materials may have been transported to the actively growing tissues. It was also possible, however, that chlorogenic acid and other compounds were synthesized in the sprouts.

Observations of seed pieces, sprouts, and young plants indicated that the phenolic materials were mobile in the vascular system of the plants. All young plants 6–10 in. tall displayed strong histochemical reactions. At this stage, relatively high phenolic concentrations could be detected throughout the seed pieces, which had shown no reaction when the sprouts were 2 in. long.

Concentrations of phenols at all the underground test points decreased as the plants developed. Fig. 1 shows the decrease during the growing season for the 6 varieties in the greenhouse. Fig. 1-A illustrates the concentrations in the root stele 2 in. from the stem.

The 3 resistant varieties maintained a concentration of 2 or more visual ferric chloride units until 160 days after planting. In the susceptible varieties Early Gem and Kennebec, the concentration was down to trace amounts at 125 days. The level in susceptible Russet Burbank was maintained between 2 and 3 units but dropped off rapidly after 125 days.

Color reactions in the underground stem node (Fig. 1-B) showed a similar decline for the 3 susceptible varieties between 125 and 150 days after planting. The resistant varieties maintained a concentration of 2 units or more beyond 160 days. Evaluations at the stem node were for both the root stele and the vascular system of the stem.

The differences between the 3 resistant and 3 susceptible varieties in reaction to ferric chloride were most readily discernible in the vascular tissues of the stem internodes (Fig. 1-C). Concentrations in susceptible Early Gem, Russet Burbank, and Kennebec decreased sharply to below 1 unit by 125 days. The resistant varieties, Populair, Great Scott, and 41956, retained concentrations of at least 2 units until 160 days after planting.

Verticillium resistance.—When resistance of the 6 varieties to Verticillium wilt (Table 1) was compared with the histochemical tests, the susceptible varieties had a symptom index from 6 to 23 at 94 days after planting, and from 66 to 95 at 115 days. Of the re-

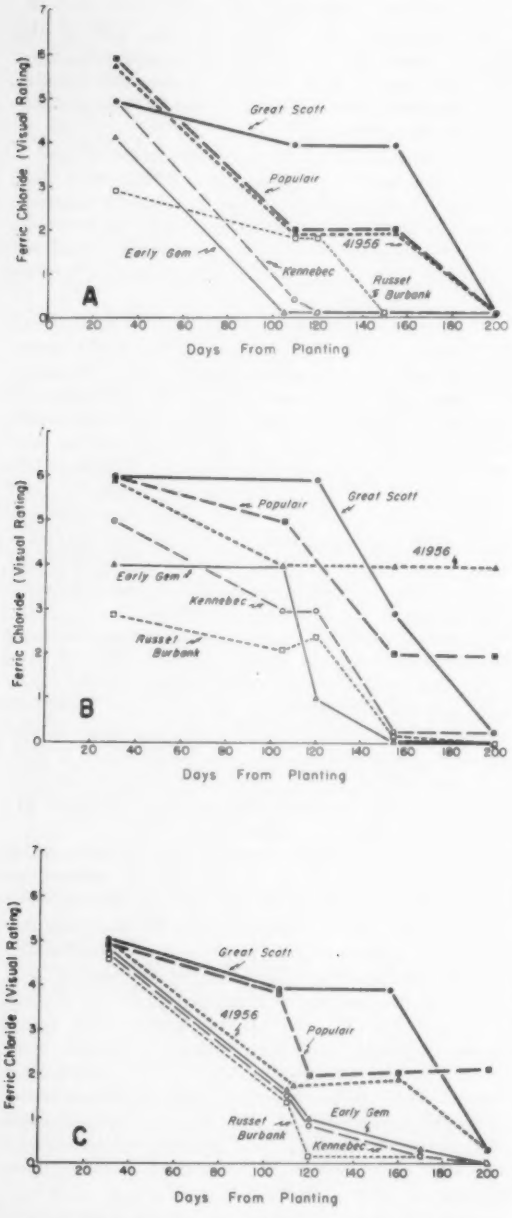

Fig. 1.—Amounts of *o*-dihydroxy phenols found during the development of 6 potato varieties as determined by the ferric chloride test. **A)** Root stele, 2 in. from stem. **B)** Underground stem node. **C)** Vascular system of stem internode.

Table 1.—Four-year average Verticillium wilt index[a] values for 3 susceptible and 3 resistant potato varieties at Aberdeen, Idaho.

Variety	Days from planting		
	94	105	115
Susceptible:			
Early Gem	23	50	95
Russet Burbank	17	32	66
Kennebec	6	38	69
Average susceptible	15.3	40.0	76.6
Resistant:			
Populair	0	0	0
41956	0	0	2
Great Scott	0	1	14
Average resistant	0	0.3	5.3

[a] Verticillium wilt index (6) includes both percentage and severity. Index values: 10, all slight; 30, all moderate; 50, all severe; 100, dead.

Table 2.—Total visual ferric chloride units of tissues at 5 locations[a] of 6 varieties of potatoes tested histochemically with 10% ferric chloride.

Variety	Days from planting				
	108	122	155	180	200
Susceptible:					
Early Gem	8	8	5	4	3
Russet Burbank	8	4	5	4	3
Kennebec	7	7	4	2	T
Average susceptible	7.7	6.3	4.7	3.3	2.0
Resistant:					
Populair	11	12	13	11	10
41956	17	15	16	12	9
Great Scott	23	23	18	11	6
Average resistant	17.0	16.7	15.7	11.3	8.3

[a] Test points were: a) root stele, b) stem node, c) stem vascular, d) stolon vascular and e) tuber vascular sections.

sistant varieties. Populair showed no symptoms, 41956 only a trace of symptoms, and Great Scott had an index reading of 14 after 115 days.

The time and severity of symptom development in the field (Table 1) and the decline in intensity of the ferric chloride reaction in the greenhouse (Fig. 1, Table 2) indicated that the decrease in concentration of phenols preceded development of the disease. A concentration of 2 or more ferric chloride test units in the root stele or at the stem node of greenhouse plants after 100 days, or in the stem vascular elements after 125 days, was highly significantly correlated with resistance in the field.

It was believed that the total concentration of phenols in the vascular system should be considered as well as the amount in any particular portion of the total visual ferric chloride units for 5 vascular test points, root stele, stem node, stem vascular, stolon vascular, and tuber vascular locations. Table 2 gives the underground plant.

The totals for the individual resistant varieties were higher throughout the growing season. The decline in the susceptible varieties resulted in 5 units or less 155 days after planting, whereas the resistant varieties maintained a total of over 10 units 180 days after planting, even though resistant 41956 and Great Scott were similar in maturity to the susceptible Russet Burbank and Kennebec varieties.

Statistical analysis of the data obtained by the ferric chloride and methyl red tests disclosed significant differences between methods, varieties, location of tests, and age of plants. Differences in intensities of the methyl red test were more difficult to determine even though the results were similar to those of the ferric chloride tests. For that reason the results with methyl red are not included.

Defoliation of plants in the greenhouse 145 and 160 days after planting delayed maturity and the decline of the concentration of phenols. In all 6 varieties, concentrations were higher for about 20 days longer than in the untreated plants.

Injecting young plants with 0.1 ml of 50 ppm of methyl ester of naphthalene acetic acid or 2,4,6-trichlorobenzoic acid delayed maturity similarly to defoliation. Injected plants also retained a higher concentration of phenols in the vascular system. Injecting young plants in the field with naphthalene acetic acid, 2,4-D, and other growth regulators also delayed maturity and the severity of Verticillium wilt symptoms.

Sections from 30-day-old plants and solutions with known amounts of chlorogenic acid were compared after treatment with ferric chloride. Ferric chloride units of 4–6 in root and stem vascular systems corresponded to 7500–10,000 ppm of chlorogenic acid. Visual ferric chloride units of 2–4 resembled concentrations of 2500–5000 ppm, whereas a trace to 2 units indicated 500-1000 ppm. No color developed when ferric chloride was added to solutions containing 250 ppm or less of chlorogenic acid. Comparisons with methyl red as an indicator produced similar results except that a faint red color could be detected at 250–100 ppm of chlorogenic acid but not at 50 ppm. When all solutions were adjusted to pH 5.0, no methyl red reaction was observed. Adjustment to pH 5.0 had no effect on the ferric chloride test.

Titration of methyl red solutions at graduated pH ranges indicated that the vascular tissues of potatoes were near pH 4.5 or below. Lowering pH values from neutral produced orange to red-orange, with a sharp break between 4.6 and 4.5 to the deep-red color of treated vascular tissue or periderm of the potato. This deep red persisted at pH below 4.5.

Spore inhibition.—Lee and Le Tourneau (4) found that *V. albo-atrum* could be grown in liquid media containing 10,000 ppm of chlorogenic acid when inoculation was made with a mycelial biscuit. The effect of different concentrations of chlorogenic acid in distilled water on individual spores was more pronounced than was mass inoculation with a mycelial biscuit.

In these trials, conidia of *V. albo-atrum* exposed to solutions of chlorogenic acid showed highly significant reductions in germination even in the low concentrations of 50 and 100 ppm (Table 3). Germination

Table 3.—Spore germination of *Verticillium albo-atrum* in various concentrations of chlorogenic acid at different pH levels.[a]

Concentrations of chlorogenic acid (ppm)	pH of solution	% germination	pH of solution adjusted to	% germination
10,000	3.3	0	5.0	0
7,500	3.7	0	5.0	0
5,000	3.8	0	5.0	0
2,500	3.9	0	5.0	0
1,000	4.0	0.7	5.0	4.0
500	4.2	4.5	5.0	6.5
250	4.5	4.5	5.0	0.0
100	5.0	6.0	5.0	4.0
50	5.5	8.5	5.5	4.0
0	7.0	27.5	7.0	28.0

[a] LSD: at .05 level, 4.08; at .01 level, 7.44.

with 1000 ppm was very slight; only spores adjacent to several mycelial fragments were capable of germination. Germination with 250–500 ppm was also better when mycelium was present. No germination occurred in concentrations of 2500 ppm or above.

At concentrations of 500 and 1000 ppm the germ tubes produced were short and frequently formed sclerotia at the apical end. At 5000 ppm and above the spores were heavy-walled after several days in the solutions, and 30–50% had no stainable cell contents.

The growth of mycelial fragments was not retarded at 50, 100, or 250 ppm. At 500 ppm no growth was found after 11 days, whereas microsclerotia were formed by the mycelial fragments in solutions of higher concentrations.

These results indicated that, though *V. albo-atrum* can be grown in concentrations of chlorogenic acid of 1000 ppm, mycelial growth was retarded at 500 ppm or above. Spore germination was inhibited by a concentration of 50 ppm.

Discussion.—Lee and Le Tourneau (4) found chlorogenic acid content was greater in the roots of 3 Verticillium-wilt-resistant varieties, Populair, 41956, and Great Scott, than in the roots of 3 susceptible varieties, Russet Burbank, Early Gem, and Kennebec. They believed that the small volume of vascular tissue compared to the larger volume of other tissues in the stem accounted for the failure to recover large quantities of chlorogenic acid (4, 14). In our studies, the use of ferric chloride as a histochemical test indicated high concentrations of *o*-dihydroxy phenols in the vascular system. Concentrations of 7500–10,000 ppm, mostly in the form of chlorogenic acid, were present in the root stele, stem node, and vascular system of young plants. Growth of mycelium or germination of spores of *V. albo-atrum* was delayed *in vitro* at concentrations below 1000 ppm of chlorogenic acid. Inhibition was complete when the concentration was maintained at 2500 ppm or higher.

Rudolph (10) described infection by *V. albo-atrum* as generally occurring through the roots, and, except for a few external lesions, confined to the vascular system of the plant. Tolmsoff and Young (12) found that the rate of symptom development, severity of symptoms, and time of death in plants of Russet Burbank were directly related to the concentration of inoculum of *V. albo-atrum*. In our trials, the higher and more prolonged concentrations of phenols within the vascular system of resistant potato varieties provided a similar method of reducing the inoculum potential by inhibiting fungus development. Inhibition or delay of the growth of *V. albo-atrum* within the vascular system would account for the delay or absence of foliar symptoms in the resistant types.

McLean (6) reported that young or actively growing plants were more resistant to Verticillium wilt than were senescent plants of the same variety. Delaying senescence by using young seed tubers (6 months instead of 12 months from planting to planting) or stimulation following defoliation delayed symptoms and decreased the severity of the disease.

A delay of 2–3 weeks in the death of the plants will reduce the effects of Verticillium wilt satisfactorily, giving a corresponding increase in potato yields in some localities (6). Symptoms can be delayed in many ways: by late planting, by use of late or resistant varieties, by planting young seed tubers, and by applying growth regulator sprays to the foliage (6, 13, 15). Growth regulating chemicals applied to whole or cut seed tubers effectively delayed Verticillium wilt but resulted in yield increases in only a few instances (7).

Inhibition of susceptibility to *V. albo-atrum* by chlorogenic acid may be similar to the mechanism of resistance to potato scab described by Schaal and Johnson (11). If the resistance mechanism is the same, inhibition would generally occur within the vascular system of the root and stem as well as delaying entry of the fungus. Further studies on plants infected with *Verticillium* might determine the role of synthesis and breakdown of chlorogenic acid on wilt resistance.

Other phenols and quinones that were inhibitory to *V. albo-atrum in vitro* delayed wilt symptoms when foliar applications were made in the field (5). The higher concentration of naturally occurring phenols in young or actively growing plants, or in resistant varieties, resulted in a more uniform and prolonged supply than could be obtained by artificial means such as sprays or injections. Thus, inhibition of *V. albo-atrum* infection would be maintained over a longer period in resistant plants.

Methyl red, used as a histochemical test, indicated a lower pH due to the presence of chlorogenic acid in certain tissues. A pH value of 4.5 or less in the vascular tissues was in agreement with Gäumann (1), who found the pH to be 4.0–4.5 in xylem and 5.6–5.9 in the pith and cortical portions of potato plants. Lee and Le Tourneau (4) did not find *V. albo-atrum* completely inhibited *in vitro* until pH was reduced to 3.2. In the present studies the effect of chlorogenic acid concentration on spore germination was not appreciably altered when pH was maintained at 5.0 or allowed to become more acid (Table 3). We believe the pH of the vascular tissue was not low enough to inhibit *V. albo-atrum*. The amount of *o*-dihydroxy phenols in the cortex of freshly harvested tubers was correlated to field resistance to Verticillium wilt with sufficient accuracy for screening large numbers of lines for resistance or susceptibility. This method could be used for eliminating many susceptible lines where field tests are impractical or unreliable. The phenolic content of the tuber cortex was generally indicative of the concentration in the vascular system of the plants. —Branch Experiment Station, University of Idaho, Aberdeen, Idaho.

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LEAF SPOT OF SESAME CAUSED BY CYLINDROSPORIUM SESAMI

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The writer thanks Dr. Charles Chupp for studying diseased leaf specimens from Florida. The sesame plantings were established in cooperation with A. J. Norden, Assistant Agronomist, Florida Experiment Station.

Cooperative investigation of the A.R.S., U.S.D.A., and the Agricultural Experiment Station, Gainesville, Florida.

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SUMMARY

Cylindrosporium sesami Hansford is established as the cause of a severe disease of sesame (*Sesamum indicum* L.) in Florida and South Carolina. This fungus has not been reported previously in the United States. The 1958 outbreak of *C. sesami* at the Florida Experiment Station in Gainesville was linked to seed-borne sclerotia, whereas local dissemination was by air-borne conidia. Growth of the fungus *in vitro* was optimum at 27±2°, slight at 16°, and nearly completely inhibited at 33°C. Sporulation was moderate on lima-bean agar and rare on potato-dextrose or nutrient agar. In the greenhouse the fungus was pathogenic to sesame and soybeans but not to tobacco and castorbeans. After inoculation of the plants in the greenhouse 18 days at about 27°C were required for lesion development. Field evaluation of the Sesame World Collection for resistance to Cylindrosporium leaf spot showed that the great majority of the material was susceptible. Certain strains that were resistant to this disease were also resistant to Alternaria leaf spot.

A severe leaf disease of sesame (*Sesamum indicum* L.) observed at Gainesville and Tallahassee, Florida, in 1958 appeared to be caused by a species of *Cylindrosporium* not previously reported in the United States. In 1959 Cylindrosporium leaf spot reappeared at Gainesville and was also found at the Sandhill Agricultural Experiment Station, in South Carolina. This disease has not been observed in Texas, where sesame is now produced commercially.

Cylindrosporium leaf spot was first described in 1861, in Uganda, and the causal fungus was named *Cylindrosporium sesami* Hansford. This disease was often confused with Cercospora leaf spot until 1936, when the Uganda Department of Agriculture published brief descriptions of both diseases (1).

The Florida occurrence of Cylindrosporium leaf spot is the subject of this paper, which deals with: 1) diagnosis of the disease in Florida, 2) spread and dissemination in Florida, 3) growth of the causal organism in culture, 4) pathogenicity of the fungus isolate, and 5) resistance of sesame.

Materials and methods.—Diagnostic material included diseased sesame leaves collected in Florida (Fig. 1-A) and South Carolina, and herbarium specimens collected in northern Nigeria and identified in 1956 by M. B. Ellis. The Nigerian specimens were provided by H. A. Dade, Commonwealth Mycological Institute, Kew, England. Type material from Uganda was unavailable.

Growth of an isolate of *Cylindrosporium* sp. from leaves collected in Florida was studied on potato-dextrose agar (PDA), nutrient agar (NA), and lima-bean agar (LBA). The influence of temperature on growth of the fungus was studied in PDA, NA, and LBA cultures incubated 35 days in 5 flask series for each medium at 16°, 22°, 33°, and room temperature (27±2°C). Growth responses were estimated visually

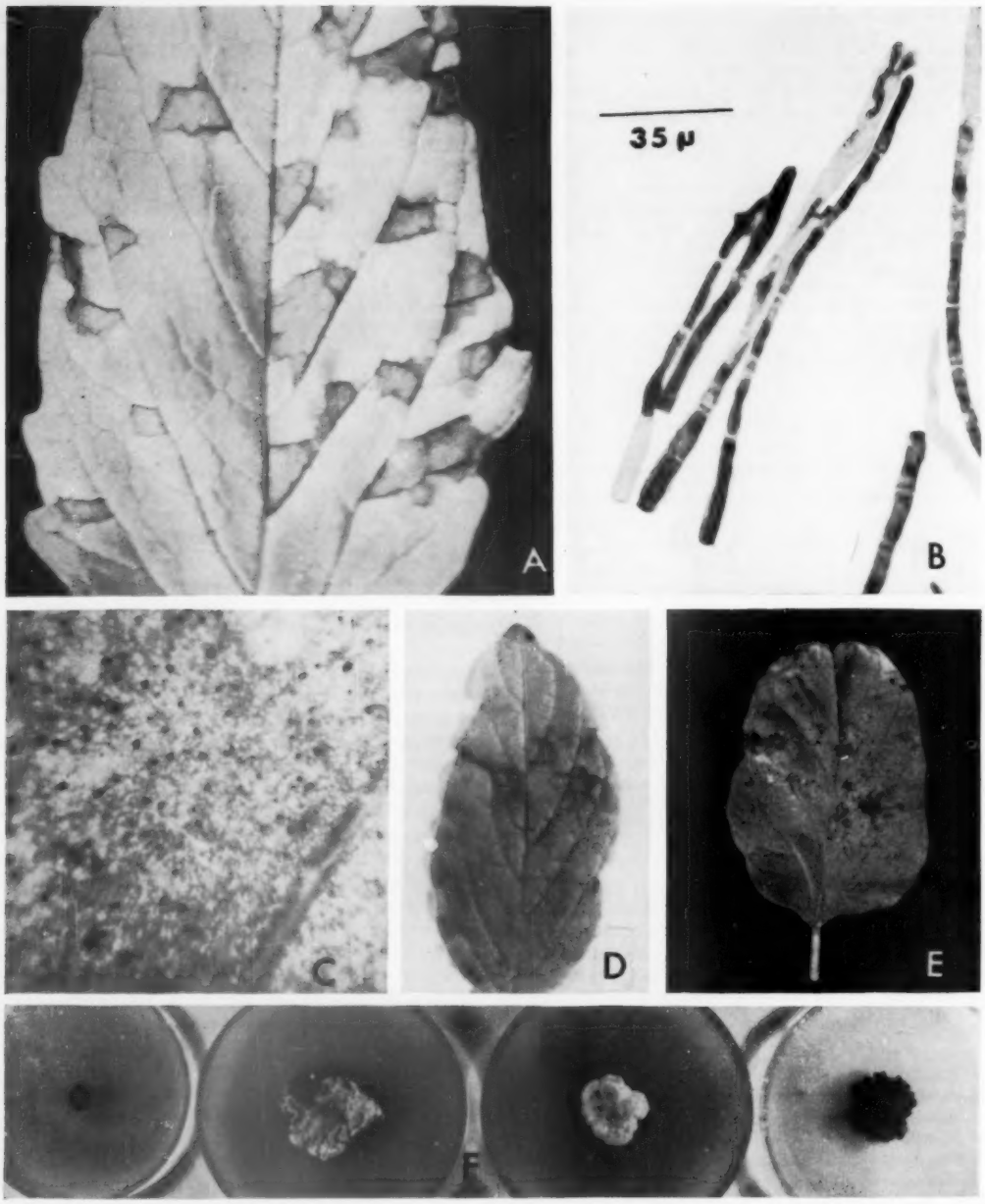

Fig. 1.—A) Symptoms of Cylindrosporium leaf spot of sesame in Florida (slightly enlarged). **B)** Photomicrograph of conidia of *Cylindrosporium sesami* stained with acid fuchsin showing unstained septa. **C)** Acervuli (35×) of *C. sesami* on the upper surface of the leaf. **D,E)** Respectively, sesame and soybean leaves inoculated with the pathogen. **F)** Growth of the Florida isolate of *C. sesami* on PDA incubated 35 days (left to right) at 16°, 22°, 27±2°, and 33°C. Mycelial growth occurred at 16° and 22°C.

as well as by detaching the colonies from their substrate and weighing them fresh.

The pathogenicity of the isolate was tested on plants atomized with water suspension of finely divided cultures grown on either PDA or LBA. The tests were made on replicated plants of Nicaragua sesame, Jackson soybean, Golden Cure tobacco, and Cimarron castorbean. The atomized plants were inclosed in polyethylene bags for 7 days and thereafter moistened daily with water until symptoms appeared. The fungus reisolated from the artificially induced lesions was identified as *C. sesami*.

Disease resistance was evaluated on sesame material planted as single rows at the Florida Experiment Station, Gainesville. In 1958, 216 lines were tested. These lines were replanted in 1959 plus 577 Plant Introduction Accessions of the Sesame World Collection, 87 advanced Texas progenies, and other miscellaneous material.

Results.—*Diagnosis of the disease.*—Diseased sesame leaves collected in Gainesville were sent to Charles Chupp for diagnosis. His report of fruiting of *Cylindrosporium* on the leaves was confirmed by the finding of subepidermal, discoid, dark acervuli, 20–50 μ in diameter, on the upper surface of the leaf (Fig. 1-C) and a few acervuli on the lower surface. The conidia (Fig. 1-B) were thread-like, curved or straight, transparent, 3-to-8-septate, on short conidiophores. Occasionally conidia scraped from diseased leaves were germinating from lateral or terminal cells. *Cylindrosporium* was isolated from leaves placed on agar medium and incubated at room temperature for 10–15 days. The mean length of 50 conidia grown on LBA at 27±2°C was 107.2 μ (standard deviation of 10.4 μ) and was slightly under that of conidia in the field.

Conidia of the Florida isolate differed slightly in length and number of septa from conidia of the isolate described by Hansford as *C. sesami* (1, 2). Lesions on Florida (Fig. 1-A) and South Carolina specimens were larger (4–10 mm) than those reported in Uganda (2–5 mm) or seen on the leaves from Nigeria (2–5 mm). The lesions on sesame leaves do not fall out and cause the "shot hole" effect, as in *Cylindrosporium* diseases of some stone fruits.

A study of the description of Cylindrosporium leaf spot and its causal organism, *C. sesami*, from Uganda (1, 2); of leaf specimens from Florida, South Carolina, and Nigeria; and of the fungus isolated from diseased leaves in Florida showed that the organism from Florida leaves was also *C. sesami*. Diseased sesame leaf specimens from Florida were deposited in the National Fungus Collections of the U. S. Department of Agriculture at Beltsville, Maryland. The Florida isolate of *C. sesami* has been assigned the American Type Culture Collection accession number 13802.

Spread and disseminaion of the disease in Florida.—In 1958 *Cylindrosporium* leaf spot first appeared in Gainesville on a row of young sesame plants originating from an untreated lot of Tall Benne seed, a local variety included as a leaf spot spreader row among other strains. The disease spread to a larger planting, comprising over 200 lines. The following season the disease recurred at Gainesville among entries of the Sesame World Collection as early as 3 weeks after emergence, and lesions continued developing until formation of new leaves ceased. The disease did not spread, however, to all material of a second planting made 12 days later about 1000 meters from the first. The circumstances of the outbreak in Gainesville and its recurrence the following season suggested that the disease was initiated from sclerotia on the Tall Benne seed and that new sclerotia that developed in the summer remained viable in the soil and on plant remains throughout the winter.

The spread of *C. sesami* during the growing season appears to be by conidia, which develop abundantly under field conditions and are disseminated into the air. Buoyancy and range of aerial dispersion and viability of the conidia of *Cylindrosporium* appeared to be limited, however, since some susceptible sesame material at the Experiment Station did not become infected in spite of its relative proximity to the infection focus.

Growth habit of C. sesami.—The fungus grew slowly on PDA, NA, and LBA at 16°, 22°, and 27±2°C. Fresh weight of the cultures was greatest on PDA and lowest on LBA. Growth at 33° was nil or slight (Fig. 1-F; 2). On these media the fungus developed first into a rough, black stromatic mass (Fig. 1-F). Development of aerial mycelium on the stromatic cultures on PDA and NA was stimulated at 22° and 27±2° but inhibited at 33° (Fig. 1-F). Mycelial fragments transferred to the

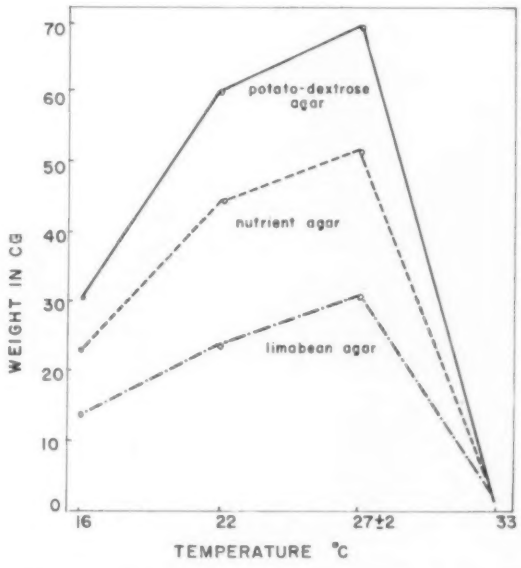

Fig. 2.—Effect of temperature on the growth *in vitro* of *Cylindrosporium sesami* Hansford.

same media produced fast-growing typical mycelial cultures, which exuded yellow droplets, sectored readily, and produced sclerotialike bodies as they aged. Growth responses of mycelial cultures to different temperatures were difficult to evaluate because of rapid growth at first and lack of clear differences at the end of the incubation period within the temperature range used.

Stromatic cultures sporulated rarely on PDA and NA but did develop conidia when the cultures were grown on LBA at 22° and 27±2°C. Agar-grown conidia differed little in length from those scraped from naturally infected leaves. No perfect stage of *C. sesami* has been found in nature or in culture.

Pathogenicity of C. sesami.—Pathogenicity tests were carried out in a greenhouse maintained near 27°C. Typical lesions developed on sesame and soybean leaves (Fig. 1-D,E) about 18 days after they were atomized with a conidial suspension. No symptoms appeared on tobacco or castorbean leaves when they were sprayed with the same conidial suspension. Inoculation of sesame and soybeans with mycelial fragments resulted in a mild blight of the terminal bud, without leaf lesions. Tobacco and castorbeans remained unaffected by inoculation with mycelial fragments of *C. sesami*. The fungus was reisolated from sesame and soybeans. The disease has not been found on soybeans in the field.

Field evaluation of sesame for resistance to Cylindrosporium leaf spot.—No strains in the evaluation at Gainesville in 1958 and 1959 were immune, but field reaction to the disease varied. The most susceptible strains were defoliated severely and died before maturity as a result of infection by both *C. sesami* and *Alternaria* sp. About 20 strains were rated as moderately resistant to both *C. sesami* and *Alternaria* sp.

Discussion.—The length and number of septa of the conidia of *C. sesami* from Florida and Uganda (1, 2) differ within the reasonable limits of variability of the species. Similarly, differences in lesion size on sesame leaf specimens in the United States and in Africa are attributed to varietal susceptibility and environmental differences.

The outbreak of the disease in Florida in 1958 and its recurrence in 1959 were linked to seed-borne sclerotia. Therefore interregional spread of the disease could be initiated by sclerotia.

The present study showed that the Florida isolate of *C. sesami* was not specific to sesame, since it caused lesions on inoculated soybeans. A field occurrence of Cylindrosporium leaf spot on soybeans is a definite possibility.

The resistance to Alternaria leaf spot of certain strains tested for field resistance to *Cylindrosporium* is promising.—A.R.S., U.S.D.A., in cooperation with the Florida Agricultural Experiment Station, Gainesville.

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ISOLATION OF THIELAVIOPSIS BASICOLA FROM BEAN RHIZOSPHERE

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SUMMARY

Small, dark-brown colonies of *Thielaviopsis basicola* were obtained from the rhizosphere of beans grown in soils artificially infested with endoconidia of the pathogen. Composition of the plating medium, kind of soil, inoculum concentration, and pathogenicity of isolates greatly influenced the rhizosphere colony counts of *T. basicola*. Media containing the antimicrobial agents oxgall and sodium propionate allowed greater numbers of colonies of *T. basicola* to develop on rhizosphere dilution plates than media without them. Colony counts were higher from beans grown in infested Berwyn loam or in a greenhouse loamy sand than from beans grown in infested compost soil or Elsinboro sandy loam. Numbers of colonies of *T. basicola* obtained were greater from the rhizosphere of beans infected with moderately to strongly pathogenic isolates than from the rhizosphere of beans infected with mildly pathogenic isolates. At least 100-1000 endoconidia per g of oven-dry soil were required to obtain infection and an appreciable number of colonies from bean rhizosphere. The pathogen could not be recovered from roots slightly diseased, or it was recovered in small numbers only. Also, it could not be recovered from soils immediately after their infestation with great numbers of endoconidia. *Fusarium solani* f. *phaseoli* in bean roots did not interfere with rhizosphere colony counts of *T. basicola*.

Early workers experienced great difficulties in isolating *Thielaviopsis basicola* (Berk. & Br.) Ferraris, the cause of black root rot of tobacco and other plants, by direct plating of infected tissue on agar media. The first successful way of obtaining pure cultures with great simplicity was isolation from soil by the carrot-disk method (12). Stover (8) isolated the pathogen from infected tobacco roots by a modification of the carrot-disk method. When macerated root material was smeared on the surface of raw potato disks the fungus grew and appeared as a grayish-white layer of endoconidia. Tsao and Van Gundy (10) failed to obtain *T. basicola* by plating infected citrus roots on agar. They were able, however, to isolate the pathogen with the "carrot-half" isolation technique, which con-

sisted of inserting surface-sterilized root pieces into narrow slits in cut surfaces of carrot roots. Ebben (3) isolated an unidentified species of *Thielaviopsis* from rhizosphere dilution plates, but not from soil dilution plates or from root tissue of tomato plants.

During experiments on the biological control of *Rhizoctonia solani* Kühn, tap roots of bean seedlings grown in greenhouse benches during winter or early spring became purple in many instances. As the seedlings grew older, coal-black lesions encircled the tap root and many of the lateral roots. Rhizosphere platings from the infected plants revealed numerous small colonies of *T. basicola* that could be counted and easily transferred, free of contaminants, to potato-dextrose agar (PDA) slants. Since isolations of *T. basicola* from infected root tissue often gave negative or variable results, the present study was conducted to determine the importance of several factors that may influence detection of this pathogen in the rhizosphere of infected bean seedlings.

Materials and methods.—Four isolates of *T. basicola* were used: isolate 1, from infected bean roots at Beltsville; isolate 2, from bean roots obtained through the courtesy of T. A. Toussoun, University of California; and isolate 8, from poinsettia roots, and isolate 10, from bean roots, both through the courtesy of M. Alexander, Cornell University. The media used were acidified PDA, dextrose-peptone-yeast extract agar (DPYA) (7), Martin's (6), Ohio (2), V-8 juice–dextrose–yeast extract agar (VDYA) (7), Warcup's (11), and modified Warcup's (7). DPYA, VDYA, and modified Warcup's medium contained, in addition to the basic ingredients, 5 g oxgall, 1 g sodium propionate, and 30 mg each of aureomycin hydrochloride and streptomycin sulfate per liter.

Rhizosphere samples were taken by removing superfluous soil from the bean roots but retaining that which adhered. Rhizosphere dilution plates were prepared by suspending 10 g of bean roots (fresh weight) in 500 ml of sterile tap water, shaking the contents for 30 minutes on a rotary shaker, diluting the resulting suspensions, and adding 10 ml of agar medium to 1 ml of the final suspension in a Petri plate. The dilutions of the final rhizosphere soil suspensions were 1:20,000. Nonrhizosphere soil was diluted by suspending the equivalent of 5 g of oven-dry soil in 500 ml of sterile tap water. Each treatment consisted of 4 replications, each replication of 6 Petri plates. Fungi were counted after incubation at 25°C for 2, 3, 4, and 6 days.

The 4 isolates used were tested for pathogenicity to beans in the greenhouse. Surface-disinfected seeds of Topcrop snap beans were planted in unsterilized soil free of *T. basicola* at the rate of 10 seeds per No. 10 tin can. Inocula of the isolates were prepared from 7-day-old cultures of the pathogen growing on PDA slants. The tubes were flooded with 15 ml of sterile distilled water, and the concentration of the endoconidia was determined with a hemocytometer. Enough spore suspension was mixed with unsterilized soil to give a known number of spores per g of oven-dry soil and to bring the moisture content of the soil to 60% of its moisture-holding capacity (MHC). The moisture content was maintained at this level by daily weighing and watering. The soils were infested at the time the seeds were planted. The temperature was maintained at 18–23°C. Except where otherwise indicated, infection was indexed 21 days after planting by rating each individual plant on an infection scale from 0 (no infection) to 4 (roots and hypocotyls completely black).

Results.—*Effect of medium on the isolation of T. basicola.*—*T. basicola* from the rhizosphere of beans grown in a greenhouse loamy sand produced the most colonies on media containing 5 g oxgall and 1 g sodium propionate per liter. For instance, *T. basicola* was not detected on acidified PDA or Warcup's medium, presumably because this slow-growing pathogen could not compete successfully with rapidly growing fungi. The pathogen was present in small numbers only on rhizosphere plates of Martin's and Ohio media (42,000 and 50,000 per g of oven-dry sample, respectively). On DPYA, VDYA, and modified Warcup's media, however, the total rhizosphere counts of *T. basicola* exceeded 500,000 per g of oven-dry sample. The small number of colonies occurring on the Ohio medium may be accounted for by the low concentration of oxgall (1 g per liter). *T. basicola* from bean rhizosphere appeared on the third or fourth day of incubation on the oxgall media as small diffuse white to gray colonies. On the sixth or the seventh day the colonies became larger (2–5 mm in diameter) and turned dark brown to black (Fig. 1). Isolations could be made by lifting the well-defined small colonies from the plate and transferring them to PDA slants.

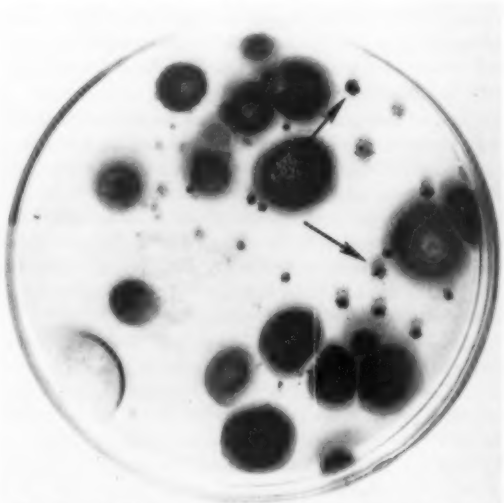

Fig. 1.—A dilution plate of V-8 juice–dextrose–yeast extract agar (with 5 g oxgall and 1 g sodium propionate per liter) showing small dark brown to black colonies of *Thielaviopsis basicola* from the rhizosphere of infected bean seedlings. Arrows indicate 2 of the colonies that can be easily isolated.

Effect of kind of soil on pathogenicity and rhizosphere colony counts of T. basicola.—Berwyn loam (pH 6.5), compost soil (pH 6.8), Elsinboro sandy loam (pH 6.3), and a greenhouse loamy sand (pH 6.2) were infested with 30,000 endoconidia from isolate 1 per g of oven-dry soil, planted with beans, and exposed to 1500 ft-c of artificial light in a growth chamber.

Root observations on 21-day-old seedlings indicated that disease severity was lower in the compost soil than in the other 3 soils. The compost soil used was made by enriching a sandy loam with organic materials and composting for 3–5 months. Disease severity was about equal in Berwyn loam, Elsinboro sandy loam, and the greenhouse loamy sand. Rhizosphere counts, however, were higher in Berwyn loam and in the greenhouse loamy sand than in Elsinboro sandy loam (Table 1). Evidently the kind of soil affected the amount of sporulation by *T. basicola* on the infected root surface.

Relative pathogenicity of 4 isolates of T. basicola and its relation to rhizosphere colony counts.—Elsinboro sandy loam and the greenhouse loamy sand were infested with 10,000 endoconidia per g of oven-dry soil from each of the 4 isolates. Although a limited number of isolates were used, it appeared that the original host of the isolate did not influence pathogenicity (Table 2). Bean isolate 1 was the most pathogenic to beans, and bean isolate 10 the least. Bean isolate 2 and poinsettia isolate 8 were intermediate in their pathogenicity to beans. All isolates retained their relative pathogenicity after they were reisolated and reinoculated into the soils.

Infection indices and rhizosphere colony counts of the pathogen were directly related (Table 2). Isolate 1 was recovered in greater numbers from the rhizosphere than the other isolates. Both disease severity and colony counts were lower in Elsinboro sandy loam than in greenhouse loamy sand.

Effect of inoculum concentration on disease severity and rhizosphere colony counts.—Aqueous endoconidial suspensions of isolates 1 and 8 were added to unsterilized Elsinboro sandy loam and to greenhouse loamy sand to give an exponential series of concentrations from 0 to 10^5 endoconidia per g of oven-dry soil. Preliminary tests suggested 90-95% viability of endoconidia from 7-day-old PDA cultures. Rhizosphere counts were made 35 days after inoculation.

A linear, though variable, increase in disease severity accompanied the exponential increase in inoculum concentration (Table 3). At least 100 endoconidia were required per g of oven-dry soil to obtain an infection index of 1.0. One and 10 endoconidia per g of oven-dry soil respectively produced no disease and disease of very low severity (infection index less than 1.0). With only a few exceptions, infection indices and colony counts were higher in the greenhouse loamy sand than in Elsinboro sandy loam. Also, in both soils, infection indices and colony counts were higher with isolate 1 than with isolate 8.

Table 1.—Numbers of *Thielaviopsis basicola* from the rhizosphere of bean seedlings grown in 4 soils.

Medium	Number[a] from rhizosphere of beans grown in specified soil[b]			
	Berwyn loam	Compost soil	Elsinboro sandy loam	Greenhouse loamy sand
VDYA without oxgall and sodium propionate	32	0	33	26
VDYA	620	5	232	389
Warcup's	34	0	0	14
Modified Warcup's	358	5	112	343

[a] Thousands per g of oven-dry sample estimated from colony counts.
[b] Infested with 30,000 endoconidia of isolate 1 per g of oven-dry soil.

Table 2.—Infection indices and numbers of *Thielaviopsis basicola* from the rhizosphere of bean seedlings grown in Elsinboro sandy loam and a greenhouse loamy sand.

Isolate	Host from which isolated	Elsinboro sandy loam[a]		Greenhouse loamy sand[a]	
		Infection index[b]	Rhizosphere number[c]	Infection index[b]	Rhizosphere number[c]
None (control)	–	0	0	0	0
1	bean	2.8	131	3.5	360
2	bean	0.8	5	2.5	44
8	poinsettia	0.9	18	2.8	51
10	bean	0.4	0	1.2	13

[a] Infested with 10,000 endoconidia of each of 4 isolates per g of oven-dry soil.
[b] Mean severity rating on individual plants on a scale of 0 (no disease) to 4 (roots and hypocotyls completely black).
[c] Thousands per g of oven-dry sample estimated from colony counts on VDYA.

After aliquots were removed from the original rhizosphere dilutions, the contents were blended for 1 minute at about 5000 rpm. From the blended gross sample, including roots and adhered soil, dilutions of 1:20,000 were prepared, and 1-ml aliquots transferred to each of 6 Petri plates. Blending the original gross sample gave a 2–3-fold increase in the number of *T. basicola* colonies on the VDYA medium. For instance, 434,000 colonies per g of oven-dry sample were estimated from the rhizosphere of beans grown in the greenhouse loamy sand infested with 100,000 endoconidia of isolate 1. Blending the original suspension resulted in 940,000 colonies per g of rhizosphere sample. Blending infected tissue was also used by Stover and Waite (9) as an improved method of isolating *Fusarium* spp.

In another experiment, endoconidia of isolates 1 and 10 were added to Elsinboro sandy loam and to greenhouse loamy sand up to 140,000 per g of oven-dry soil. Immediately after spores and soils were mixed thoroughly, soil dilutions of 1:5,000 were prepared. In no instance could *T. basicola* be recovered from the infested soils by the dilution plate method.

Root rot severity and rhizosphere colony counts from a mixture of 2 root rot fungi.—Although the black root rot of beans and the dry root rot (caused by *Fusarium solani* f. *phaseoli* (Burk.) Snyd. & Hans.) differ in the symptoms they produce and in the time these appear in the field, black root rot is frequently associated with the dry root rot on beans (1). Because of the frequent association of the 2 pathogens it was desirable to find out whether the rhizosphere colony counts could reveal the presence of *T. basicola* in bean roots infected with both pathogens. Unsterilized root-rot-free greenhouse loamy sand was divided into 4 portions. The first portion served as control. The second and third portions were respectively infested with endoconidia of *T. basicola* and with macrospores of *F. solani* f. *phaseoli* (8000 spores per g of oven-dry soil). The fourth portion was infested with a mixture of the 2 pathogens (4,000 spores of each per g of oven-dry soil).

Table 3.—Effect of inoculum concentration on infection indices and numbers of *Thielaviopsis basicola* from the rhizosphere of bean seedlings grown in Elsinboro sandy loam and a greenhouse loamy sand.

Soil and endoconidia added per g of oven-dry soil	Isolate 1		Isolate 8	
	Infection index[a]	Rhizosphere number[b]	Infection index[a]	Rhizosphere number[b]
Elsinboro sandy loam				
0	0	0	0	0
1	0	0	0	0
10	0.2	0	0.1	0
10^2	0.9	6	0.7	0
10^3	1.3	63	1.2	0
10^4	3.5	72	3.0	40
10^5	4.0	305	3.5	83
Greenhouse loamy sand				
0	0	0	0	0
1	0	0	0	0
10	0.6	0	0.3	0
10^2	1.0	86	1.2	16
10^3	2.6	285	1.9	30
10^4	3.6	256	2.3	65
10^5	4.0	434	4.0	211

[a] Mean severity rating on individual plants on a scale of 0 (no disease) to 4 (roots and hypocotyls completely black).
[b] Thousands per g of oven-dry sample estimated from colony counts on VDYA.

Table 4.—Infection indices and numbers of fungi from the rhizosphere of bean seedlings grown in a greenhouse loamy sand infested with *Thielaviopsis basicola*, *Fusarium solani* f. *phaseoli*, or both.

Treatment	Infection index[b]	Rhizosphere number[a]		
		Total fungi	*T. basicola*	*Fusarium* spp.
Uninoculated control	0	810	0	40
F. solani f. *phaseoli*	1.6	1,498	0	90
T. basicola	3.2	1,087	642	39
F. solani f. *phaseoli* plus *T. basicola*	3.1	2,890	1,910	93

[a] Thousands per g of oven-dry sample estimated from colony counts on VDYA.
[b] Mean severity rating on individual plants on a scale of 0 (no disease) to 4 (roots and hypocotyls completely black).

The results (Table 4) indicated that *T. basicola* could be detected on rhizosphere plates from beans grown in the 2 portions of soil that were infested with this pathogen, irrespective of the presence or absence of *F. solani* f. *phaseoli*. Counts of *Fusarium* spp. from the rhizosphere of beans grown in the 2 portions of soil infested with *F. solani* f. *phaseoli*, in the presence or absence of *T. basicola*, were twice as high as the counts from the portions of soil not infested with *F. solani* f. *phaseoli*. No attempt was made, however, to identify the *Fusarium* spp. on the dilution plates.

The presence of *F. solani* f. *phaseoli* did not influence the severity of the black root rot (Table 4). Burkholder (1) made similar observations on the same disease complex. The dry root rot was not as severe as the black root rot. Evidently the temperature of incubation (20–23°C) was responsible for less disease caused by *F. solani* f. *phaseoli* than by *T. basicola*, since the *Fusarium* isolate used was highly pathogenic to beans in other experiments not described in this report.

Origin of colonies on dilution plates.—The origin of colonies of *T. basicola* from the rhizosphere was determined by transferring 1-ml portions from the final dilutions to the surface of a thin layer of the Ohio medium in Petri plates. Microscopic examinations of the surface of the agar on the third day of incubation revealed that the majority of colonies originated from endoconidia from the infected roots. In several instances, however, the colonies originated from chlamydospores seen to germinate on the agar surface. Additional microscopic examinations established the existence of both endoconidia and chlamydospores of the pathogen on the infected bean roots.

Discussion.—Oxgall and sodium propionate were very effective in restricting colony size of rapidly spreading fungi and thus facilitating enumeration and isolation of great numbers of colonies of *T. basicola* from the rhizosphere of infected beans. In fact the inclusion of oxgall and sodium propionate in the medium was the critical factor for revealing the presence of *T. basicola* on rhizosphere dilution plates. The effectiveness of these 2 antimicrobial agents was expected, since their use for isolating fungi has been suggested previously (2, 5, 7).

The successful isolation of *T. basicola* from rhizosphere dilutions and the failure of isolation from dilutions of artificially infested soil indicated either that its spore concentration per g of oven-dry rhizosphere sample was considerably higher than its artificial concentration (140,000 endoconidia per g) in the unplanted soil or that the endoconidia added to unplanted soil were subjected to some factor or factors that prevented their immediate recovery by the soil dilution method. It is also possible that incubation and/or bean roots are necessary to obtain a concentration of endoconidia high enough to permit recovery

on dilution plates. The lack of colonies of *T. basicola* on soil dilution plates could not be attributed to antagonistic effects of other fungi, since the slow-growing *T. basicola* appeared on rhizosphere dilution plates among many other fungi.

The effects of kind of soil and quality and concentration of inoculum on rhizosphere colony counts of *T. basicola* were perhaps of secondary importance, since the same soil and inoculum factors that increased disease severity were also responsible for great numbers of colonies of the pathogen on rhizosphere plates. Evidently soil and inoculum conditions that increased disease severity also enhanced the formation of endoconidia and chlamydospores of the pathogen in the bean rhizosphere.

No attempts were made to find out why disease severity was lower in Elsinboro sandy loam and especially in compost soil than in Berwyn loam and in the greenhouse loamy sand. All soils were maintained at 60% of MHC in all experiments. In no instance could fluctuations in disease severity and rhizosphere colony counts be attributed to soil reaction. In fact, the compost soil, with a reaction close to neutrality (pH 6.8), was more "resistant" to establishment of the disease than was the greenhouse loamy sand, with a slightly acid reaction (pH 6.2). If soil reaction was the sole factor influencing disease severity and recovery of *T. basicola* from the host rhizosphere, this would seem to contradict Johnson and Hartman's findings (4) that alkaline soils are more favorable to the disease than acid ones.

The fact that the pathogen could not be recovered from roots with mild infection may limit the scope of the rhizosphere dilution plate method of isolation. This limitation may be partly offset, however, by blending the original gross suspension. Also significant may be the fact that rhizosphere colony counts of *T. basicola* are not affected by the presence of *F. solani* f. *phaseoli*. The possibility of recovering *T. basicola* from bean rhizosphere despite the presence of other root rot organisms may be useful in establishing the presence of this pathogen in the roots of several plants in which mixtures of root rot fungi occur.—Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

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CAUSE AND PREVENTION OF THE RAPID SPREAD OF THE ASCOCHYTA DISEASE OF CHRYSANTHEMUM

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SUMMARY

Observations on the Ascochyta disease of chrysanthemum, caused by *Mycosphaerella ligulicola* (*Ascochyta chrysanthemi*), indicate that the recent alarming spread of this disease was entirely unnecessary and that further spread can be prevented. For over 40 years after its discovery, in 1904, the disease was practically restricted to the Southeastern states. The fungus spread rapidly in the last decade, during which the southern chrysanthemum industry expanded and began shipping both flowers and cuttings to other areas in quantity. Once established in an area, the fungus is both difficult and costly to eradicate, and control of the disease remains as an additional production expense. This is because of several recent developments: General adoption of year-around flowering of chrysanthemums continuously presents the host in its most susceptible form. Air-borne ascospores from neighboring plantings, or ascospores and conidia from previous plantings in the same field, may infect each successive crop. The common infection of stems, leaves, and roots reported here, with or without ray blight of flowers, facilitates persistence of the fungus and its spread with cuttings, and reduces the chance of fungicidal control.

The fungus is able to develop under a wide range of environmental conditions. Although moisture is necessary for infection, pycnidia and perithecia are developed in infected tissue under very dry conditions (even at 6% RH). In dry areas the importance of the disease is limited unless overhead watering is practiced, but the fungus may persist for indefinite periods. Under favorable conditions the fungus has a very high biotic potential. The key to the prevention of further spread is the distribution of pathogen-free stock, produced only in areas distant from other commercially grown chrysanthemums, in a program involving soil treatment with heat or chemicals, use of clean planting stock, destruction of all infected material over a considerable area, frequent application of fungicides, and avoidance of overhead watering.

The chrysanthemum has long been one of the three most important floricultural crops in the United States, and now is possibly the first crop in wholesale value (6) because of its rapid increase in popularity as a pot plant, cut flower, and garden ornamental. The Ascochyta disease of this plant, caused by *Mycosphaerella ligulicola* K. Baker, Dimock, & L. H. Davis (*Ascochyta chrysanthemi* Stev.), has spread alarmingly and become increasingly destructive in commercial cultivation during the last decade. The disease, commonly known as ray blight, may well appear in other areas in the next few years. This is completely unnecessary, since further spread can easily be prevented. Because of the severe damage caused by the disease, and because control methods are difficult and expensive, pathologists and growers in all countries should be aware of the seriousness of the situation and should take steps to prevent establishment of the disease in their areas. Therefore, this paper brings together recent information on the disease, supplements it with some new data, and indicates what can be done to prevent further spread. The observations reported here that were made in New York and Florida were by Dimock, those in California were by Baker and Davis.

Occurrence of the disease.—During the last 10 years a portion of the chrysanthemum industry has transferred from the Northeastern to the Southern states, where the Ascochyta disease has long been important. Recently, too, cuttings for propagation have been extensively grown in the south and shipped over much of the United States. Still another factor that has contributed to the increasing importance of the disease is the development of year-around flowering of the crop, continuously presenting the host in its most susceptible form.

The fungus was first observed causing ray blight of chrysanthemum flowers in 1904 in North Carolina (12). This phase of the disease was also reported in South Carolina in 1912 and Mississippi in 1932, and has continued to be important in those areas (2). The fungus was identified by H. C. Young on commercial chrysanthemums in Lake County, Ohio in October, 1923, according to information supplied by H. A. Runnels, Ohio Agricultural Experiment Station, Wooster. This may have been the basis of the record in the Weiss index (14, p. 169). There were no subsequent records of the fungus in that state until 1959. That the fungus apparently did not spread and establish out of the Southeastern states in more than 40 years was probably due to the following facts: 1) propagative material was shipped into, but not out of, that area; 2) almost no cut flowers were shipped from there into Northern states; 3) the crop was of limited importance in the South. In the late forties, however, many wholesale growers began to ship flowers from Florida to northern markets, and by about 1957 a few propagators were shipping cuttings from Florida to other regions. The chrysanthemum industry in Florida experienced an astonishingly rapid development, from a planting of 4.6 A. in 1949–50 to well over 300 A. in 1958–59 (11). The ray blight fungus has been present in that state at least since 1948 (2).

The period of spread.—The Ascochyta disease was observed in 3 commercial plantings in Florida in 1954. In propagation beds with overhead misting the fungus rotted the growing point of the cuttings and decayed the leaves, forming pycnidia in the tissues. If planted, such cuttings often sprouted from below. Affected cuttings usually occurred in small patches, the cuttings at the center being rotted, those at the margin showing only infected leaves and some stem lesions. The fungus was also abundant in the stock-plant beds. In spite of regular spraying with zineb (zinc ethylenebis[dithiocarbamate]), flowers of standard varieties commonly rotted in plantings with overhead watering. The petals decayed at the base and fell from the receptacle. The disease was serious on the East Coast of Florida in plantings grown from locally propagated stock, but did not occur at that time on the West Coast, where cuttings were shipped in from Northern states.

Coincident with the development of the chrysanthemum cutting and cut-flower industry in Florida and elsewhere in the South, the fungus began to spread. It appeared in destructive form in Prince Georges County, Maryland, in 1947 (2), probably brought in on cuttings from Montgomery, Alabama. For the first time outside of the United States, the disease was reported in Japan in 1952 (10, p. 44), and appeared in 2 areas of New South Wales in 1955 (4). It was first observed in New York in a field planting on Long Island in 1956 (8), the following year in Seneca County, in 1958 in Wayne County, and in 1959 in Tompkins, Broome, and Erie Counties. Because of the exceptionally wet summer of 1958 the disease became very serious in New York, particularly on the standards, Indianapolis White and Indianapolis Yellow, but was also common on pompons. It was observed by J. Tammen (Department of Botany and Plant Pathology, Pennsylvania State University, University Park) in Cumberland County, Pennsylvania, in 1956, and in Columbia and Centre Counties in 1959 (personal communication). The Ascochyta disease was found by plant pathologists of the National Agricultural Advisory Service and the Glasshouse Crops Research Institute in several nurseries in southern England in 1959 on plants grown from cuttings imported from the United States. The crown phase occurred in one in-

stance, leaf rot in another, and flower and bud infection was also seen in some cases (personal communication from M. Hollings, Glasshouse Crops Research Institute, Littlehampton). It was reported (personal communication) in Denver County, Colorado, in 1959 by R. R. Baker (Department of Botany and Plant Pathology, Colorado State University, Fort Collins). In Ohio the disease was found by C. J. Olson (Yoder Brothers, Inc., Ashtabula, Ohio) in 1959 (personal communication). In each instance, once introduced, the fungus caused severe losses at frequent intervals.

The authors reported (2) in 1949: "There apparently is nothing in the climate of the eastern, midwestern, and northwestern growing areas that would check the disease, once it was introduced." "Fore-warned, fore-armed" is valid only if the warning is heeded. This appears to be yet another instance of a well-studied pathogen needlessly distributed by man, even though known to be potentially dangerous to an important crop over a wide area.

New symptoms produced in California.—Both the Ascochyta and Mycosphaerella stages were observed on dead bases of commercial chrysanthemums in San Mateo County, California, in 1949 (2), but the fungus was not found again in that state, nor did it cause disease until 1959 (1).

In April, 1959 in a commercial planting under plastic in Los Angeles County, the varieties Albatross and Good News were severely affected by a type of symptom not previously reported. Cuttings had been purchased from an eastern propagator and increased in blocks for planting the beds. In both propagation and flower-production beds the fungus had developed abundantly, causing stem cankers. Some of these lesions started at the cut ends of stems up to 5 in. above the soil (Fig. 1-B), some at the point of attachment of leaves decayed by the fungus (Figs. 1-C, E), some at the point where a leaf base was penetrated by a young root (Fig. 1-D), and others at the base of the cutting. The cankers were irregular in shape, up to 1 in. long, reddish brown at first, then deep brown to black. Pycnidia were frequently observed in these cankers, and in a few cases perithecia as well. In beds planted for flower production the disease was primarily a basal stem canker (Fig. 1-E) that caused stunting of the plant. This was sometimes evident as a crippled condition of the shoot tip, with small leaves and bending to one side. Some of these plants showed no stem cankers, and the condition may have been due to some other factor. The fungus sometimes grew from the stem into the roots, causing a brown decay.

Irregular, soft, dark brown blotches up to 1 in.

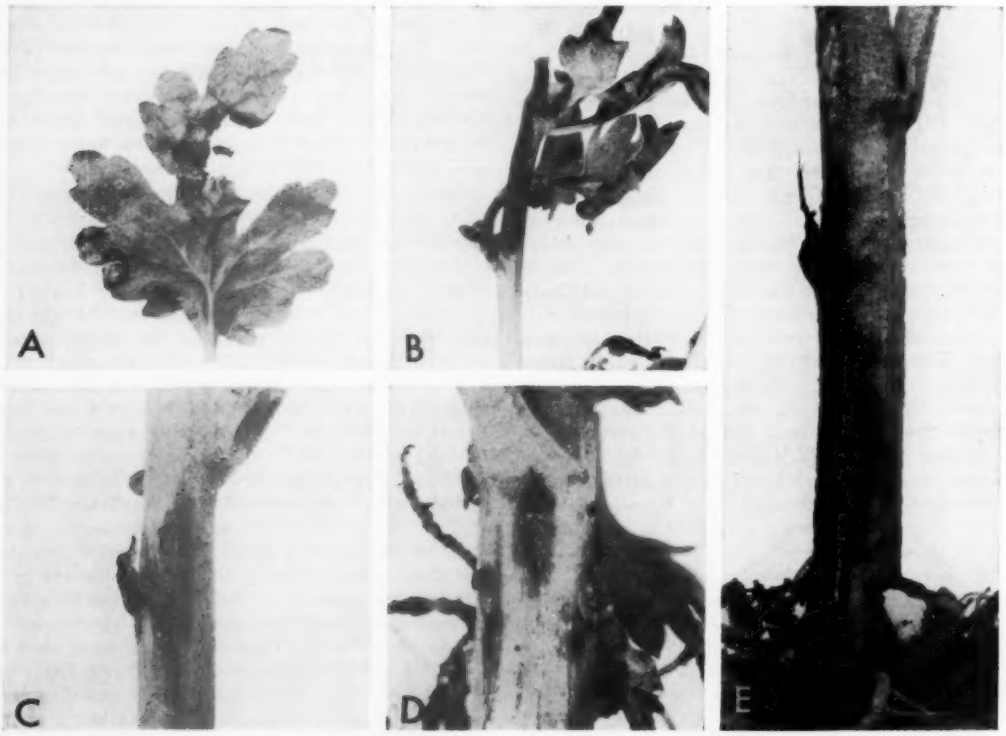

Fig. 1.—Symptoms produced by *Ascochyta chrysanthemi* on vegetative parts of chrysanthemum. **A)** Leaf decay, showing pycnidial formation and falling away of tissue. **B)** Stem decay starting at the cut end of a stem where a cutting was removed. **C)** Stem invasion by the fungus from a rotted leaf. **D)** Stem decay starting at wound resulting from penetration of leaf base by a developing root. **E)** Typical basal stem canker formed by fungus advance from a rotted leaf.

across, bearing pycnidia and having areas partially rotted away (Fig. 1-A), developed on leaves, particularly near the soil. Similar leafspots were reported earlier in North Carolina (2) and New South Wales (4).

The fungus was readily isolated from stem, leaf, and root lesions, and conidia from subcultures were used to inoculate flowers of the varieties Albatross, Grand Slam, Whitetop, Prince, and Fortnighter in the laboratory. (Flowers for these inoculations were supplied by the Department of Floriculture and Ornamental Horticulture.) Typical tiny spots developed (pink on the white varieties), advancing to petal decay on all but the last variety, which was somewhat more resistant than the others. In morphology and pathogenicity the California fungus is the *Ascochyta chrysanthemi* known in the Southeast.

The spectacular nature of a ray-blight outbreak tends to direct attention away from the stem and leaf phases of the disease. Perhaps partly because of this, it had not previously been appreciated that the fungus could also seriously injure the growing plant. The stem and leaf phases increase the potential damage, ensure carryover of the fungus, and facilitate its spread with cuttings.

In the fall of 1959 the same stem and leaf symptoms were found by A. H. McCain and R. D. Raabe (Department of Plant Pathology, University of California, Berkeley) in a commercial planting in Alameda County, California, but the ray-blight phase of the disease still has not positively been identified as occurring naturally in California. The grower in Los Angeles County spoke of pink spots on the flowers at an earlier date, but none were seen by the writers. It is probable that ray blight will become important in the arid Southwest only with overhead irrigation.

The appearance of the disease on stems and leaves is sufficiently similar to injury caused by Botrytis or bacterial blight that confusion has undoubtedly arisen in some cases. The production of ethylene by Ascochyta-infected host tissue (9, 16) causes early senescence of nearby leaves, particularly in bundles of flowers during marketing. Evidence now indicates that infection by the fungus is favored by such senescent tissue or by wounds, although flower petals are easily penetrated, a situation occurring also with *Botrytis cinerea* Pers.

Development of the fungus under dry conditions.—In semi-arid California, chrysanthemum plants remain dry except during brief periods of rain or overhead sprinkling, and the fungus must then grow intermittently. This it is able to do (2). As indicated earlier, the fungus is able to form pycnidia and perithecia under very dry conditions. It was desirable to determine how dry the tissues could be without preventing formation of perithecia and pycnidia.

Flower heads of the variety White Snow were inoculated with dense conidial suspensions of *A. chrysanthemi* and held in the laboratory in moist chambers. The fungus advanced down the flower pedicels, forming long girdling lesions. After 12 days the infected pieces of the stem were cut out and dried for 3 days by exposure to laboratory air. They were then cut into 2-in. lengths and tied with stainless-steel wire into small bundles of 6–8 stems. There was no evidence of either pycnidia or perithecia on the stems selected for use. These bundles were fastened at the top of sealed 2-qt fruit jars each containing 1 qt of a mixture of sulfuric acid and water adjusted to provide a different RH (relative humidity) (13), as indicated in Table 1.

Table 1.—Development of perithecia and pycnidia on infected chrysanthemum stems held at different relative humidities for 18 weeks.

	Stems held at 50°F		Stems held at 70°F	
Approx. RH	Perithecia	Pycnidia	Perithecia	Pycnidia
100[a]	−	−	−	−
85[a]	−	−	+	+
72	+	+	+	+
60	−	+	+	+
48	−	+	−	−
38	+	+		
29	−	+	−	+
21	+	+	−	−
12	−	+	+	+
6	+	+	+	+
2			−	−

[a] Stems overgrown with saprophytes at these humidities.

The jars were placed in the water of constant-temperature tanks (set for 50 and 70°F) in the glasshouse. This provided diffuse daylight and normal day length for the experimental period (February to June). After 18 weeks the stems were removed and carefully examined under a widefield microscope. Although the results (Table 1) do not give precise information on growth at different humidities, they show that the fungus is able to develop the imperfect and perfect stages from living mycelium in dried stems at RH levels as low as 6%. This is a lower RH than that to which the fungus would be exposed in California, and the performance is consistent with its survival there in the absence of disease expression (2).

A second test was set up in the same way, using the white pompon variety, Arcadia. The stems were dried in the laboratory and held over mixtures of sulfuric acid and water to give approximately the same RH levels as before. The jars were held 28 weeks in constant temperature cabinets in the dark at 50 and 70°F. No perithecia were formed in any series, but pycnidia were formed in all series except those overgrown at high humidities and those at 2% RH. It is possible that perithecia did not form in this test because the stems were held in the dark.

Control of the disease.—Control of the Ascochyta disease in most areas is now considerably more difficult than it was in 1949 (2). The fungus is well established in a number of important northern commercial growing areas, where, in the judgment of the authors, it can be eradicated only at great expense and inconvenience. Hence every feasible precaution should be observed to prevent further introductions.

The disease probably will remain as another costly production complication, thus removing one of the few remaining advantages of northern growers over those of the Southern states. This is due to at least 4 factors: 1) Perpetuation of the fungus in an area is favored by the general adoption of year-around culture, since ascospores and conidia may be spread to each successive crop of cuttings from the preceding one. 2) Since ascospores may be carried from infected stock perhaps as much as a half mile away, eradication of the fungus in a given planting requires either elimination of other chrysanthemums in a large area or concurrent ray-blight control in these plantings, as well as effective destruction of carryover inoculum by chemical or heat treatment of the soil to be planted, and the use of cuttings free of the fungus. 3) The tendency of the fungus to form basal stem cankers which may extend below the soil surface, makes improbable the successful prevention of infection by any fungicide application. The number of infections would be greatly reduced, but the fungus would persist in the planting as a potential threat. 4) The fungus is now known to cause serious losses in nonflowering blocks and propagation beds, as well as in cutting benches, without occurrence of the ray-blight phase of the disease. Furthermore, the symptoms produced so resemble those caused by Botrytis or bacterial blight that the Ascochyta disease may be recognized only after the fungus is well established.

Fungicidal application.—This type of control is preventive or suppressive but does not eliminate the pathogen, and is therefore used to supplement other methods. Stock plants in mother blocks should be sprayed thoroughly every 7-14 days with ferbam (ferric dimethyldithiocarbamate) at 1 lb wettable powder per 100 gal. or perhaps maneb (manganous ethylenebis [dithiocarbamate]) or zineb at 1 lb per 100 gal. A suitable spreader must be used to ensure coverage. These sprays should continue in the cutting bench, where, if overhead misting is used, the frequency of application must be increased. When cuttings are planted in beds for flower production ferbam sprays should be continued until the flower buds begin to develop. At that time applications of zineb or captan (N-(trichloromethylthio)-4-cyclohexene-1.2-dicarboximide) at ½–¾ lb commercial wettable powder per 100 gal. should be started. A suitable wetting agent should be added. Application should be made with a multiple-nozzle boom that delivers a fine mist spray from above. The objective is to protect flowers from the ray-blight phase. During wet weather 2–3 applications per week may be necessary, and in an epidemic area or season daily applications may be required. With proper equipment applications may be made very rapidly. It is not known whether adequate control of either the ray-blight or stem and leaf phases can be obtained by fungicidal dust applications.

Sanitation.—So great is the biotic potential of this fungus that practices aimed at reduction of initial inoculum must be followed if success is to be obtained. As a routine control all infected plants should be destroyed by burning or burying. Less satisfactory is deeply plowing the refuse under in the field, because some parts of the stems will remain exposed and start new infections from discharged ascospores or splashed conidia. Preferable is the treatment of field soil with chloropicrin (trichloronitromethane) under a plastic tarp, as practiced for control of Verticillium wilt in California (15), or by steam, as used for nematode control in Florida (3). Wholly unacceptable is the placing of diseased plants or flowers on refuse piles, since ascospores from them may reinfect the clean planting. Growing a crop other than chrysanthemum in a 2-year rotation is useful if the field is sufficiently far from current plantings to limit ascospore infections.

In glasshouses and in raised beds under plastic, the soil should be treated with steam or chloropicrin between successive plantings.

In gardens, covering the unopened buds with plastic bags has prevented infection. The bags should be left open at the bottom to reduce condensation inside.

Pathogen-free stock.—Chrysanthemum cuttings, more than the propagules of most other important vegetatively propagated crops, are produced by a few specialist companies. One of these alone produced over 100 million cuttings in 1958. As long as this situation exists, the possible threat of explosive spread of the pathogen is always present; more importantly, however, it provides a mechanism for preventing any further dissemination. With proper care and vigilance, the producers of cuttings can keep their chrysanthemums free of the ray-blight fungus. These propagators have, for the most part, accepted their responsibility of providing the floricultural industry with pathogen-free cuttings. Indeed, the rapid decrease in importance of Verticillium wilt (7) and stunt virus disease (5) of chrysanthemum is largely due to their efforts.

As has been pointed out, the ray-blight fungus is very difficult to control once it is established. It is clear that the recent spread of the pathogen has been largely by infected cuttings. The control of this disease is logically through the production and distribution of pathogen-free stock, and the propagators have an obligation to supply such cuttings. If they fail to do so, the important position of the chrysanthemum in the floricultural industry may be definitely weakened.

Commercial cutting production is seriously complicated by the presence of the ray-blight fungus. To produce cuttings in an area near which the fungus is developing is almost certain to result in infected or infested material, and the recent rapid spread of the fungus appears to have been largely due to this practice. Thus propagation specialists should not be in an area closely surrounded by commercial chrysanthemum growers. Neither should cuttings be taken from beds or houses where the disease is known to occur. A propagator should have no difficulty in producing Ascochyta-free cuttings under glass or plastic in an area that is isolated, provided he maintains a rigorous program of inspection, sanitation, soil treatment, fungicide application, and watering by means other than overhead.

The flower grower who purchases his cuttings should make certain that the supplier meets the above requirement. Those producing their own propagative stock should take tip cuttings only from healthy plants grown under glass or rainproof plastic cover, without overhead watering, and should institute the program outlined above.—Departments of Plant Pathology, University of California, Los Angeles, California, and Cornell University, Ithaca, New York.

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FACTORS AFFECTING POPULATION LEVELS OF CRICONEMOIDES XENOPLAX

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SUMMARY

Thompson seedless grape is a good host for *Criconemoides xenoplax*, and Lovell and S-37 peach are poor hosts. A soil temperature of 26°C favors increase of *C. xenoplax* more than 13, 18, 21, or 28°C. No difference was detected between pH 5 and pH 7 in effect on population levels of *C. xenoplax* on peach. In a lath-house pot test, peach seedlings were not injured by *C. xenoplax* at population levels as high as any yet found in California peach orchards.

Introduction.—The ectoparasitic nematode *Criconemoides xenoplax* Raski, 1952, is widely distributed in North America (1, 2, 7, 8, 9, 11, 12) and Europe (13). It is pathogenic to carnation, *Dianthus caryophyllus* L. (11) and parasitic on grape *Vitis vinifera* L. (10, 13), peach, *Prunus persica* (L.) Batsch (12), plum, *Prunus domestica* L. (4), and elm, *Ulmus americana* L. (1). It has been associated with walnut, *Juglans* sp. (8), pine *Pinus* sp. (8), California laurel, *Umbellaria californica* (H. & A.) Nutt. (8), and maple, *Acer* sp. (2).

An attempt to study the pathogenicity of *C. xenoplax* to peach (7) disclosed a dearth of knowledge of the host preferences and ecological requirements of this nematode. The present paper reports on a study of the effects of soil temperature on *C. xenoplax* parasitizing Lovell peach, S-37 peach, and Thompson seedless grape, and the effect of soil pH on *C. xenoplax* parasitizing the two peach varieties.

Materials and methods.—S-37 and Lovell peach seedlings, and Thompson seedless grape cuttings, grown 6 weeks in sand and watered with one-half-strength Hoagland's nutrient solution (5), were transplanted to a well-mixed, sandy peach orchard soil (moisture equivalent, 4.4) containing about 600 *C. xenoplax* per 250 cc (1.7 per g). *C. xenoplax* was the predominant nematode species in the soil, and the only species whose population changes were followed.

The temperature test was conducted in 5-in. clay pots supported on coarse gravel inside glazed crocks immersed in a constant-temperature water bath. This arrangement allowed less precise soil temperature control than could have been obtained by planting directly into the crocks, but it provided the soil drainage necessitated by condensation at the lower temperatures. Seven replicates of each of the 2 peach varieties, and of grape, and 2 fallow replicates were maintained 4 months at each of 5 temperatures.

The pH experiment, conducted in 6-in. clay pots sunk in soil beds in an outdoor lath-house, was during Oct.–May. The peach seedlings were dormant during

the 3 winter months. To estimate pH × *C. xenoplax* interaction as it affected plant growth, an ethylene dibromide treatment was included: 6cc Dowfume W85 (83% ethylene dibromide by weight) per 30 gal. of the mixed orchard soil in a closed 55-gal. drum. The factorial design of the experiment included ethylene dibromide and no treatment, Lovell and S-37, soil pH 4.7 and 7.3, with 8 replications of each of the 8 possible combinations of these pairs.

Plants in both the temperature and the pH experiments were watered with one-half-strength Hoagland's nutrient solution (5). The soil pH resulting was 7.3±0.2 (the mean, with its standard error, of 4 pH meter determinations from 4 pots of each of the 2 pH series; determinations were made at the end of the 7-month test). A pH of 4.7±0.4 was achieved in one-half the pots in the test of pH effect by using one-half-strength Hoagland's solution whose pH had been lowered to 3 with 38% (by weight) hydrochloric acid.

Determining the viability of *C. xenoplax* is a very subjective procedure because of the sluggish nature of this species. Therefore, counts of *C. xenoplax* were of total larvae and females—live or dead. Males of this species are uncommon, and were not counted. Eggs were not recovered or counted. At the end of the experiments *C. xenoplax* was recovered from a 250-cc aliquant of well-mixed soil from each pot. A modification of Cobb's (3) screening-gravity method was used, involving screens of 20, 100, 100, 200, and 200 mesh in a racked (6) series. Contents of all but the 20-mesh screen were saved. Nematodes attached to the root system were recovered by washing the roots over the screen series with a forceful stream from a spray nozzle. The total numbers of *C. xenoplax* recovered from the soil and from the roots were calculated after counting the *C. xenoplax* in aliquots from the screenings and multiplying by appropriate factors. These 2 totals were then combined to obtain the number of *C. xenoplax* per pot.

Fresh weights of each plant and root were obtained after blotting each root system dry.

Results.—*Plant effects.*—When the experiments were terminated *C. xenoplax* was observed in feeding position on the roots of the 2 peach varieties, and on the roots of Thompson seedless grape. Mean numbers of *C. xenoplax* per pot, and per gram of root, were strikingly higher ($P = <0.01$) for grape than for the peach varieties, or the fallow. Thompson seedless grape is evidently a good host for *C. xenoplax;* Lovell and S-37 peach were relatively poor hosts.

The mean number of *C. xenoplax* resulting with the culture of peach variety Lovell did not differ significantly from the mean for variety S-37.

The initial mean number of *C. xenoplax* in all pots, and the final mean numbers for the peach varieties, were all significantly higher ($P = <0.01$) than the final mean number of *C. xenoplax* in the fallow treatment (Fig. 1).

In the 4-month temperature test, final population levels of *C. xenoplax* showed little or no increase on peach (Fig. 1). Seven months' growth of the peach varieties in the pH test increased ($P = <0.01$) the number of *C. xenoplax* to 3–6 times the initial level (Fig. 2).

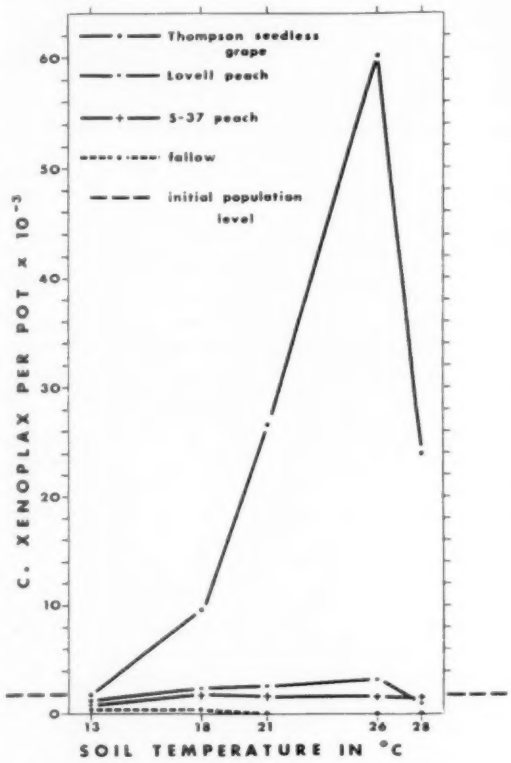

Fig. 1.—Final mean numbers per pot of *C. xenoplax* at 5 soil temperatures after 4 month's growth of Thompson seedless grape, Lovell peach, and S-37 peach, and after fallow.

Temperature effects.—Final populations with Thompson seedless grape provided the only sensitive measure of temperature effects on *C. xenoplax*. Of the soil temperatures tested, 26°C produced the greatest increase in nematode population (Fig. 1: LSD between mean numbers for grape at the 1% level is 23,590). Final numbers of *C. xenoplax* were positively correlated with root weights in this test ($P = <0.01$). When final populations of *C. xenoplax* on grape were expressed in terms of nematodes per gram of root, 26°C remained the highest mean ($P = <0.01$). No interpolation along connecting lines in Fig. 1 is warranted.

pH effects.—Final mean numbers of *C. xenoplax* per pot, or per gram of root, at pH 5 did not differ significantly from the corresponding means at pH 7. Mean weights of peach seedlings were less ($P = <0.01$) from acid Hoagland's solution than from normal Hoagland's solution. This was true for peaches in both untreated and ethylene-dibromide-treated soil.

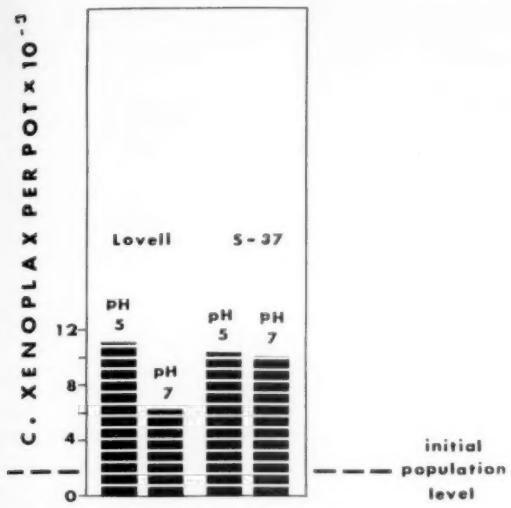

Fig. 2.—Final mean numbers of *C. xenoplax* per pot after 7 months' growth of Lovell and S-37 peach at two soil pH levels. (No significant differences between means; all higher than the number of *C. xenoplax* initially present—$P < 0.01$.)

(The pH × soil treatment interaction in terms of plant weights was not statistically significant.)

Ethylene dibromide effects.—No *C. xenoplax* were recovered from the pots representing the ethylene-dibromide-treated soil. Mean weights of peach seedlings did not differ significantly between treated and untreated soil.

Discussion.—Sher (11) increased *C. xenoplax* from 100 to 12000 on carnation in 3 months. With Thompson seedless grape, Raski and Radewald (10) increased 100 *C. xenoplax* to over 6000 in 5 months. The lesser (3-fold) increase on peach noted by Thomas (12), and the zero to 6-fold increases on peach in the present study make it apparent that peach is a comparatively poor host for *C. xenoplax*. Carnation or grape would be more sensitive hosts than peach for further tests of ecological effects on the nematode.

Since *C. xenoplax* reached a higher population level at 26°C than at the lower temperatures tested, moisture fluctuation appears a more likely cause than high temperature for summer reductions in population levels of *C. xenoplax* in Merced Co. (California) peach orchards (7).

The lack of peach growth response to ethylene dibromide soil treatment indicates that *C. xenoplax* caused no appreciable injury to peach at the population density (1-7 *C. xenoplax* per gram of soil) at which it occurred in this experiment. This population level is as high as any recorded in California peach orchards to date.

Thus far, the only plant on which *C. xenoplax* has been demonstrated to be pathogenic is carnation (11). —Department of Plant Nematology, University of California, Davis.

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A COMPARISON OF VIRUS ISOLATES, DWARF-FRUIT AND SPY 227-LETHAL

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SUMMARY

The only difference distinguished between Spy 227-lethal virus and dwarf-fruit virus inoculations was the less drastic effect of Spy 227-lethal virus on Hyslop fruit. Inoculation with both isolates resulted in stem pitting of Hyslop, chlorotic leaf spot and leaf distortion of the R12740-7A indicator variety, and, through short-term observation, leaf mottling of Spy 227. Neither produced symptoms of mosaic or rubbery wood on Lord Lambourne variety in 2 years in the field. The spread of both diseases to adjacent trees after long-term association indicates natural root-graft inoculations.

Introduction.—A disease of apple, since named "dwarf fruit and tree decline," was observed in 1944 under conditions that demonstrated its virus nature (1, 2). The only virus disease then listed for apple was apple mosaic (11). It seemed apparent that other anomalies reported for apple were also caused by viruses. One such anomaly was the killing of Spy 227 rootstock grafted to a normal appearing selection of McIntosh (4, 9, 10). The similarities between this and the dwarf fruit disease included latency in some varietal sources and tree decline on special indicators. The present study compares these diseases on common indicators.

Materials and methods.—In 1945, two rows (A and B), 16 ft apart were planted to Hyslop trees spaced 6 ft apart in the row. Nine alternate trees in Row A were budded or grafted with a source of Jonathan from dwarf-fruit-diseased trees. The buds and grafts were placed in the top branches. Tissue union was successful in all cases, but since only a few of the buds or grafts were allowed to grow, the main branches were predominantly Hyslop.

In 1949, when the trees were 5 years old, three alternate Hyslop trees in Row B were inoculated with a source of McIntosh designated as Mac 106, carrying the factor later called Spy 227-lethal virus. Three other alternate Hyslop trees were inoculated to a source of McIntosh not lethal to Spy 227 (10). The sources of McIntosh were obtained from New York through the cooperation of H. B. Tukey.

Bud inoculations with Hyslop carrying the Spy 227-lethal virus were also made in the field on two Spy 227 trees in 1958 and two more in 1959. Similar inoculations of Hyslop buds from tree A-1 carrying the dwarf fruit virus were made on four Spy 227 trees in 1959. Comparative inoculations from the Spy 227-lethal and dwarf-fruit viruses were made in the field on 3 Lambourne trees each in 1958. This particular Lambourne clone, obtained indirectly from England via Purdue University, has since been established as an authentic rubbery wood indicator, and it has proved a good indicator for apple mosaic in other tests at East Lansing. Ten potted Spy 227 and more than 10 each of Russian 12740-7A trees, all on seedling roots, were likewise comparatively inoculated with the two isolates in the greenhouse in February, 1960. The 12740-7A clone was obtained from J. R. Shay, Purdue University. This clone originated at the University of Illinois from seed of *Malus pumila* Mill. obtained from Russia. It was used in scab resistance studies (3) and later as an indicator for a chlorotic leaf spot reaction that demonstrates at least one frequently latent virus of apple (8).

Results.—Table 1 shows the location of the Hyslop trees planted in 1945, inoculations made, trees showing fruit and stem-pitting symptoms, and the longevity of inoculated trees.

Table 1.—Inoculations, symptom production, and natural spread of dwarf fruit and Spy 227-lethal viruses on Hyslop.

ROW A				ROW B			
Tree	Inoculation 1945[a]	Fruit Symptoms	Stem Pitting	Tree	Inoculation 1949[a]	Fruit Symptoms	Stem Pitting
1	D	+ '49	+	22	O	—	—
2	O	+ '59	+	23	O	—	—
3	D	+ '49	+	24	O	—	—
4	O	+ '59	+	25	O	—	—
5	D	+ '49	+	26	O	—	—
6	O	—	—	27	O	—	—
7	D	+ '49	out '55	28	O	—	—
8	D	+ '49	out '55	29	O	—	—
9	O	+ '54	+	30	O	—	—
10	D	+ '49	out '56	31	O	—	—
11	O	—	—	32	M	—	—
12	D	+ '49	out '54	33	O	—	—
13	O	—	—	34	M	—	—
14	D	+ '49	out '54	35	O	—	—
15	O	—	—	36	M	—	—
16	O	—	—	37	O	+ '58	+
17	D	+ '49	out '53	38	SL	+ '53	+
18	O	—	—	39	O	+ '58	+
19	O	—	—	40	SL	+ '53	+
20	O	—	—	41	O	—	—
21	O	—	—	42	SL	+ '53	+
				43	O	—	—

[a] D = dwarf fruit inoculation; O = not inoculated; SL = Spy 227-lethal McIntosh inoculation; M = Spy 227-non-lethal McIntosh inoculation.

The nine trees in Row A inoculated with the dwarf fruit isolates in 1945 had shown typical dwarf-fruit symptoms by 1949. The Hyslop fruit was smaller than normal, oblate, distinctly truncate at the calyx end, and more or less deeply five-lobed (Fig. 1). Tree decline, shown by slower growth, became increasingly evident with the passing years. Six of the nine inoculated trees died 8–12 years after inoculation. All of the uninoculated trees remained uniformly healthy and vigorous. After 15 years the three surviving inoculated trees are conspicuously smaller than normal, averaging 3 in. in trunk diameter, compared with the 6-in. average of the normal trees.

Fig. 1.—Comparison of typical Hyslop fruit resulting from Spy 227-lethal (Mac 106) and dwarf fruit inoculations.

The first fruit symptoms resulting from the Spy 227-lethal inoculations were observed in 1953, the third succeeding growing season. The inoculated trees produced fruits mildly misshapen compared with fruit from normal trees. The symptoms were more subtle and less prominent than with the dwarf-fruit disease, the fruits being less indented, less truncate at the calyx end, and less reduced in size (Fig. 1). This difference between the Spy-lethal and dwarf-fruit diseases has remained constant. The stunting of Hyslop trees was not pronounced following the Spy 227-lethal (Mac 106) inoculations, which may possibly result from the advanced maturity of the trees when inoculated.

Both apple diseases showed some spread in the field, but only to adjacent trees. The naturally infected trees next to trees inoculated with Spy 227-lethal virus showed the milder fruit effects of that disease. Likewise, the trees close to the dwarf-fruit-inoculated trees showed the more severe dwarf-fruit symptoms. The spread of dwarf-fruit virus was first noticed after 12 years, whereas Spy 227-lethal virus spread was noticed in the 9th year. The infection of only immediately adjacent trees indicates inoculation by natural root grafting.

The first examination for stem pitting was in 1959. Both the Spy 227-lethal and dwarf-fruit disease isolates incited on Hyslop severe stem pitting similar to or identical with that reported on Virginia Crab (6). The pitting was observed only on the trees also showing fruit symptoms. Pitting was evident at ground level and on limbs at eye level. All the other trees showed smooth stems when the bark was peeled. This agrees with the report (7) that Hyslop shows stem-pitting symptoms when inoculated with certain varietal sources. Stem pitting and leaf distortion are shown on a Hopi crab seedling in Fig. 2-A,B.

Isolates of the two apple diseases reacted similarly

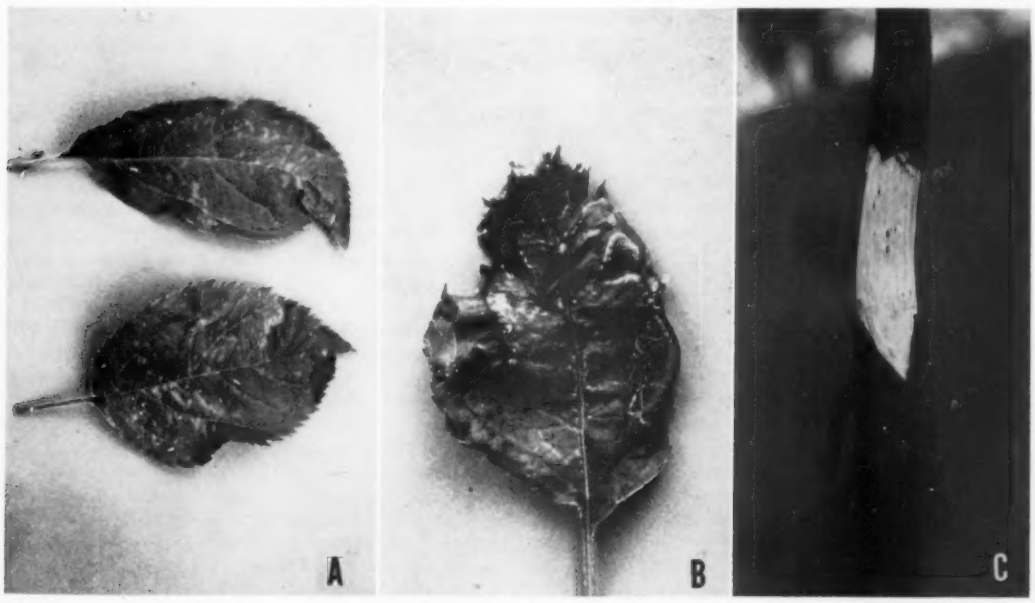

Fig. 2.—A) Mottling of Spy 227 leaves typical of Spy 227-lethal and dwarf-fruit inoculations. **B,C)** Typical distorted leaf with necrotic margin and stem pitting of a Hopi seedling inoculated with dwarf-fruit disease.

on potted R12740-7A trees inoculated in the greenhouse by chip budding at bud break. Shoots first showed a decided bending at the tip (epinasty), an unequal, unilateral development of leaves, and chlorotic leaf mottle. This is the chlorotic leaf spot syndrome reported by Mink and Shay (8).

Both the Spy 227-lethal virus and dwarf-fruit virus isolates produced less drastic immediate reactions on Spy 227 trees in the greenhouse than on R12740-7A: faint chlorotic leaf spots at 3 months (Fig. 2A). Both have produced similar mottling on Spy 227 leaves in the field the year following inoculation and also during the second year for the Spy 227-lethal inoculation, which has been observed longer.

Discussion.—The difference between the two isolates in degree of fruit dwarfing of Hyslop and the similarity of symptoms on other indicators suggests a number of hypotheses. The isolates could be carrying separate virus entities, strains of the same virus, or a different complex of viruses.

The rapid lethal effect of McIntosh isolates reported on Spy 227 (4, 5, 9, 10) was not observed in this study. This effect would perhaps be more evident and rapid when virus-carrying scions are propagated on own-rooted Spy 227 rootstock in the nursery.

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SYMPOSIUM ON SCLEROTIUM ROLFSII

INVITATIONAL PAPERS PRESENTED FEBRUARY 1-3, 1960, AT THE MEETING OF THE SOUTHERN DIVISION AT BIRMINGHAM, ALABAMA

SUMMATION

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The first record of a disease caused by *Sclerotium rolfsii* Sacc. was the description of tomato blight by Peter Henry Rolfs, in Florida in 1892. The fungus nature of the disease was determined with some difficulty, as indicated by this note: "In June 1892 I discovered the cause of the disease, not however, until my ingenuity and patience had been exhausted." Nearly 20 years later, in 1911, Saacardo named the fungus *Sclerotium rolfsii*, in recognition of Rolfs' pioneer work —and no doubt his patience, too.

Curzi, in 1931, proposed the generic name *Corticium* for the fungus, based on studies of the perfect stage in pure culture. When West, in 1945, found the fungus sporulating in nature on climbing fig, he suggested that the generic name be changed to *Pellicularia* since it was characterized by aerolate hymenia, short-celled, stout hyphae, and right-angled branching of mycelia.

The fungus has been reported from the tropics and the warmer parts of the temperate zones throughout the world. In the United States nearly every southern state from Florida to California has recorded its presence, and there is one report from Canada. The few records from Europe include Italy, Germany, and Russia.

The most extensive host list includes 189 species distributed in 8 monocotyledonous and 42 dicotyledonous families. This list was dated 1931, however, and numerous new hosts in many plant families have been recorded since that time.

Cardinal temperatures for growth of the fungus in pure culture are 8°, 30–35°, and 40°C. The vegetative hyphae do not stand freezing and longevity of sclerotia is increased by cool dry air.

The fungus will grow in beef-extract-peptone broth medium initially adjusted from pH 1.4 to 8.8, although after 6 weeks' growth the final pH is near 4.0, irrespective of the initial pH. It is able to utilize numerous simple and complex carbohydrates, and adding a sugar to a proteinaceous medium usually results in a 10-fold increase in weight of mycelium. Oxalic acid and various oxalates are produced in pure culture, and Higgins demonstrated that these compounds would cause plant injuries. The fungus also produces a wide array of enzymes. It grows and produces sclerotia on a synthetic medium but is unable to synthesize thiamine. Nitrites inhibit growth at 20 ppm and completely suppress it at 400 ppm.

Extreme variations in morphological characteristics have been noted in world-wide collections of the pathogen. Single-basidiospore isolates, however, from a single hymenium frequently show the range of variation for the entire species. Isolates from a given area are frequently fairly uniform, and this fact, according to Cooper, may be an expression of dominance in the dicaryon. Homothallism has been demonstrated by Lyle and Mundkur, but variations in single-basidiospore isolates may indicate heterothallism. Other differences in the growth responses of various isolates have been noted, and differences in isolate virulence have been shown by using tolerant to resistant test plants, although host specialization has not been demonstrated conclusively.

Losses caused by *Sclerotium rolfsii* to sugar beets were reduced by Leach with heavy fertilization with nitrogen. In a greenhouse study of major fertilizer elements, by Hudgins, nitrogen was also the only element that reduced blight on peanuts. These results were interpreted as an effect on the host that permitted recovery from lesions initiated early in the life of the plant. Boyle emphasized that inorganic salts in themselves have no direct effect on the pathogenicity of the organism. It appears from the available evidence that the role of fertilizer elements is not completely understood.

The growth of the fungus in nature is nearly always near the soil surface. This has been interpreted as indicating a high O_2 requirement. It has been demonstrated in pure culture, however, that it will grow equally over a wide range of O_2 concentration. Because contaminating molds outgrew *Sclerotium rolfsii* in atmospheres of low oxygen concentration, Flados concluded that the latter was more prevalent in the upper few inches of soil because the density of competing or antagonistic organisms was greater at lower soil levels. Boyle agreed that aerobic factors are coincidental and cannot be taken as basis for control. He stated that the organism works near the surface in heavy soils and at greater depths in lighter soils.

The ecological basis of control is given in early papers by Higgins and Taubenhaus in which it was shown that sclerotia buried over 3 in. deep were in-

nocuous. Watkins labeled *Sclerotium rolfsii* a facultative parasite that grows well on plant debris found in most soils of fairly low fertility. Boyle described the fungus as necrotrophic, i.e., it prefers dead food, and he has shown that the sclerotium does not contain sufficient energy to establish a pathogenic relationship between fungus and host. If suitable substrata are available in a favorable environment, vegetative growth is rapid and enough metabolites are produced to cause death of host tissue and eventually the host itself. Boyle also suggests that since the organism is necrotrophic the susceptibility of live hosts has little relation to their value as a medium for the production of sclerotia—so there is little opportunity to reduce the amount of inoculum by rotation. This view is not reinforced by Cooper's data, summarized by Garren, in which disease incidence in peanuts was reduced by rotations. Two means by which organic matter is introduced into new infection zones are: 1) defoliation, and 2) cultural practices that involve piling soil around the base of plants. This concept was first developed by Ciccarone and Platone, in Venezuela in 1949. Boyle, in 1952, presented data showing that soil preparation to bury all undecayed organic matter and plant debris at least 3 in. significantly increased yield and reduced infection by *S. rolfsii* when compared to soil preparation by discing in which the undecayed organic matter and plant debris were mixed in only the top layer of soil. Similar experiments, extending over 5 years in Virginia, were reported by Garren. Two methods of weed control: 1) conventional cultivation ("dirting"), and 2) chemical weed control combined with flat cultivation ("nondirting") were superimposed on the deep-burial and the surface-mulch soil preparation treatments. Disease was always maximum in surface-mulching-"dirting" plots. The higher the level of infection, the greater the benefits to be expected from nondirting and deep burial. Of these two practices nondirting cultivation contributed most to reducing infection.

The role of antagonists is disputed. Boyle feels that, until more evidence shows that antagonistic organisms are far more universal in importance, the odds are against such a popular explanation. Reports have been presented, however, that indicate that antibiosis does occur, and Watkins believes there is much evidence for the exceptional importance of antagonistic organisms in the soil.

Varietal resistance has been demonstrated in a number of hosts. It has been attributed to an impervious cuticle, thick-walled cortical cells, cork cambium activity, etc. Resistance in *Lycopersicon pimpinellifolium* is a monofactorial dominant, and in all others is either multifactorial or unknown. Varietal immunity in a species is unknown.

No real progress was made with chemical control until the advent of pentachloronitrobenzene (Terraclor), which has been used recently on ornamentals, vegetables, and peanuts with varying success. It is recommended for use on Dutch iris, peanuts, and peppers in several states. In other crops, e.g., tomatoes, the safety range is apparently very narrow.

Cooper reported good results since 1956 with Terraclor in control of *Sclerotium rolfsii* in peanuts in North Carolina. In large-scale commercial tests in Texas, Terraclor applied at planting time increased yields and grade even where levels of infection were low. Garren, in contrast, obtained increases in yield with Terraclor in Virginia only where "dirting" cultivation was used. He considered that there was no basis for recommending Terraclor either as a supplement or substitute for nondirting cultivation. A summary of data from 5 southern states indicates that Terraclor is more effective against southern blight when used alone than when applied with gypsum.

The 68 years since Peter Henry Rolfs first described how he "caught" the fungus in pure culture have seen the publication of some 700 or 800 papers on the subject. Nevertheless the plant pathologist in 1960 will too often find appropriate the words Rolfs used in describing surveys made at the urgent request of growers: "In most cases the work of destruction had been done, and nothing was left to do but record the fact."

SCLEROTIUM ROLFSII, HISTORY, TAXONOMY, HOST RANGE, AND DISTRIBUTION

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The original description of this interesting but infamous fungus, giving it an official scientific name, was published by P. A. Saccardo (8), in 1911. Its history began at least 20 years earlier, as evidenced by articles of Peter Henry Rolfs in the bulletins and annual reports of the Florida Agricultural Experiment Station. Very few other publications dealt with this organism in the period between Rolfs' writings and Saccardo's publication. There is little doubt that the organism existed in many other parts of the United States and the tropical world. This supposition is borne out by the large number of publications on the subject that appeared from many of the warmer parts of the world in the years following publication of the technical name. The history of *Sclerotium rolfsii* and the disease it causes has become quite voluminous during the past 30 years. Present interest indicates that the literature on the subject will continue to grow.

Dr. Rolfs (7) recognized the small, round sclerotia of his fungus as the outstanding morphological characteristic of the organism. In recognition of Rolfs' under-

standing that the fungus belonged to the group of non-spore-producing fungi designated Sclerotium, Saccardo honored him by naming the organism *Sclerotium rolfsii* sp. n. Variations in the appearance of the mycelium and in the size, color, and configuration of the sclerotia have been noted from time to time, but none have proved definite enough or consistent enough to merit separation as a species or variety. Welch described *Sclerotium delphinii* in 1924, but the consensus among mycologists relegates this to the status of a form at the most.

From 1930 to 1934 several people were working with a perfect state developed in cultures of *Sclerotium rolfsii*, and five published articles on their findings: Goto (3), in Japan; Curzi (2), in Italy; Milthorpe (5), in Australia; Barrett (1), in the United States; and Mundkur (6), in India. All agreed that the perfect stage was a *Corticium* sp. *Corticium centrifugum* (Lév.) Bres. was considered to coincide most nearly with the characteristics of the new fungus, in color and thickness. Since the new fungus was known only on culture media, differences in character between this and naturally grown hymenia were not considered comparable. As a result, the name *Corticium rolfsii* (Sacc.) Curzi, proposed by Curzi in 1931, was generally adopted.

The status of the name of the fungus remained unchanged for several years, and no new efforts are known to have been made to connect the vegetative stage with any sporulating phase. In 1945 and again in 1946, West (10), in Florida, found the fungus growing in abundance on a hitherto unreported host, *Ficus pumila* L. Further observations when sunny weather followed a rainy period revealed the basidial stage covering the undersides of the leaves. Measurements of the basidia and spores matched those published for *Corticium rolfsii* (Sacc.) Curzi. Since *Corticium* species having an areolate hymenium, short-celled, stout hyphae, right-angled branching of the mycelium, and stout basidia have been segregated in the genus *Pellicularia*, the combination *Pellicularia rolfsii* (Sacc.) West was proposed as the correct name for the fungus. No additional information on the taxonomy has been published.

The host range of the vegetative stage of the fungus is unusually wide. Rolfs, in his early writings, listed 15 host plants he had observed, including weeds and garden plants, some of which were shrubby. G. F. Weber (9), in 1931, listed 189 host species from many different plant families covering a fern family and 8 monocot and 42 dicot families. Since then, many new hosts have been recorded in publications from many parts of the warm temperate and tropical regions around the world. Many of these hosts are crop plants of great economic importance, but many other groups, such as garden ornamentals and weeds, are susceptible. A great majority of species on the host list are annuals or herbaceous perennials, but some woody plants are attacked as young plants. No more extensive list of hosts has been published since the article by Weber, and no complete world-wide listing has been made of the species parasitized.

The geographical distribution of this organism can best be visualized by considering published records of its occurrence. With few exceptions these are in countries in the warmer parts of the temperate zone, and in the tropics. In the southern United States it has been reported from almost every state from Florida to California. There is one report from Canada.

In South America it has been recorded from Argentina, Brazil, Colombia, British Guiana, Surinam, and Trinidad. There are many reports from the West Indies, including Dominican Republic, Bermuda, Barbados, Jamaica, St. Vincent, Puerto Rico, and Cuba.

The few records from Europe include Italy, Germany, and U.S.S.R.

Africa is represented by Egypt, Tunis, Gold Coast, Sierra Leone, Gambia, Belgian Congo, Uganda, Southern Rhodesia, Nyssaland, Madagascar, and Union of South Africa.

A number of articles report its occurrence in Asia, such as Iran, India, Japan, Malaya, Indo-china, Ceylon, and Formosa.

In the Pacific area it has been found in the Philippines, Java, Sumatra, and Hawaii. Several of the states of the Australian Commonwealth also mention its appearance within their limits.

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PHYSIOLOGY OF SCLEROTIUM ROLFSII, WITH EMPHASIS ON PARASITISM

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In each of the past few years, 15–35 papers and reports have dealt with *Sclerotium rolfsii* Sacc., evidence of the interest in the fungus and the infections associated with it. Most of those reports were casual accounts of occurrence, but an occasional one was of more fundamental nature. From that literature the present reviewer has considered the items that pertain to the physiology of the fungus, for presentation here as a basis for understanding the aspects of parasitism exhibited.

Two principal general studies have been published (5, 7). From these we know the responses of the mycelium to temperature and soil moisture, something about survival of the mycelium, the production of oxalic acid by the fungus, and some microscopic aspects of host-parasite relations. From other works has come an understanding of the nutritional requirements of *S. rolfsii*, dormancy in the sclerotia, oxygen in relation to hyphal growth, production of extracellular enzymes, and other relevant information.

According to Higgins (7), the mycelium of *S. rolfsii* grew at 8–40°C, and grew well at 20–37°C. Minimum, optimum, and maximum temperatures for growth were respectively 8°, 30–35°, and 40°C. Vegetative hyphae did not stand freezing, being killed by 24 hours at −2°C. Mature ungerminated sclerotia survived 48 hours at −10°C. These findings are confirmed. The broad range of temperature for optimum growth permits luxuriant development of the mycelium in most soils warm enough to encourage the growth of warm-season crops.

Epps et al (5) examined the interrelated effects of atmospheric humidity and temperature on the survival of vegetative mycelium that had been air-dried in the autoclaved seeds on which the fungus had grown. Four levels of relative humidity (RH) were studied (12, 25, 37, and 50%), and five levels of temperature (7–8°, 20°, 27°, 32°, and room temperature, which ranged from 20° to 30°C). Mycelia stored at 50% RH and 32°C generally failed to survive a month, whereas those stored at 12 or 25% RH and 7–8°C were vigorous after a year. Cool, dry air thus seems related to the longevity of vegetative mycelia.

In a study of the influence of soil temperature on the infectivity of mycelia the same workers reported a combined average of 3.01 days from inoculation to death of young plants of assorted dicotyledonous species at 35°C, an average that increased steadily to 6.26 days with decline of temperature to 15°C.

Higgins (7) studied the response of *S. rolfsii* to hydrogen-ion concentration in pure cultures on a liquid substrate. Although different isolates varied slightly, the fungus grew in beef-extract-peptone broth cultures initially adjusted to a series of levels over the range from pH 1.4 to 8.8. Final levels after 6 weeks of mycelial growth tended to be near pH 4.0. His examination of substrates involved the addition to a basal beef-extract-peptone medium of various compounds: sucrose, glucose, lactose, glycerine, starch, oxalic acid. Studied as possible substrates were peptone, mannite, citric acid, albumin, and skim milk. The addition of the sugars resulted, in most cases, in more than a ten-fold increase in weight of mycelium developed. Higgins also studied some of the metabolic by-products in such cultures and became impressed by the ubiquity of oxalic acid and oxalates among the staling products in cultures in which reasonable mycelial growth had occurred. In several series of experiments he showed that such culture filtrates, as well as aqueous solutions of oxalic acid, ammonium oxalate, or urea oxalate, when applied to plant tissues, brought about injuries similar to those induced by the mycelium itself.

Joham (9) studied factorially the nutritional requirements of *S. rolfsii*, and formulated therefrom an optimum complete synthetic medium that would support abundant hyphal growth and the formation of sclerotia. Yields were maximum on the following solution: 0.0015*M* $MgSO_4$, 0.004*M* K_2HPO_4, 0.002*M* KCl, 0.0125*M* NH_4NO_3, 0.1 ppm thiamine chloride, 4.0% glucose, and 2.0 ppm each of iron, zinc, and manganese.

The inability of *S. rolfsii* to synthesize thiamine from simple substances was reported in 1938 by Robbins and Kavanagh (17), who found that of the two thiamine intermediates, pyrimidine and thiazole, the former is effective in promoting normal growth, but the latter is not. Kavanagh (12) showed later that the fungus synthesizes thiazole, which it combines with externally supplied pyrimidine to form thiamine, which functions in the metabolism of the fungus. From the relative weights of mycelium developed in cultures containing thiamine and thiamine plus thiazole, Kavanagh concluded that *S. rolfsii* also destroys thiazole.

Joham (9) attempted to determine the level at which thiamine was required, but found that the lowest level at which he incorporated the vitamin in the medium, 0.1 ppm, gave as abundant growth as did any of the higher concentrations. It appears that *S. rolfsii* has the ability to synthesize from simple ingredients in the substrate all other accessory growth compounds it may require.

In an effort to determine the ability of *S. rolfsii* to use a number of simple and complex carbohydrates as sole sources of carbon and also as sources auxiliary to glucose, Johnson and Joham (11) added these materials variously to Joham's basal medium and measured the dry weights of mats developing on appropriate aliquots. The carbohydrates used were glu-

cose, mannose, fructose, galactose, sucrose, maltose, cellobiose, lactose, raffinose, dextrin, starch, inulin, pectin, pectic acid, cellulose, agar agar, gum mastic, gum guaiac, gum arabic, gum tragacanth, arabinose, xylose, and rhamnose. Of these, cellulose, agar agar, gum mastic, gum guaiac, and rhamnose were not used by the fungus. All the remainder were used with more or less efficiency, as was castor bean globulin (a protein), as sources of carbon. Johnson and Joham suggested that *S. rolfsii* is able to secrete a considerable array of exogenous carbohydrases and that those may play an important part in the the parasitic action of the fungus.

Following the lead suggested by the outcome of field experiments with nitrogenous fertilizers reported by Leach and Davey (13), in which some reduction in losses from southern blight occurred, Johnson (10) studied effects of high concentrations of nitrite, nitrate, and ammonium compounds on the growth of mycelium *in vitro*. Nitrites began to inhibit the fungus at 20 ppm, and completely suppressed it at 400 ppm. Growth in ammonium nitrate was maximum at 16,000 ppm, in sodium nitrate at 16,000 ppm, and in ammonium sulfate at 36,000 ppm. Johnson said that *S. rolfsii* tolerated calcium as calcium chloride at 3000 ppm before an inhibition was noted. Calcium at 4000 ppm suppressed growth. Such levels are far above the concentrations of calcium available even in highly calcareous soils.

The role of calcium in this connection has intrigued several investigators. Menon (14) attempted to grow the fungus on samples of Houston black clay, a highly calcareous soil in which *S. rolfsii* lacks pathogenic importance in nature. In parallel series of autoclaved and unautoclaved soil, the fungus grew readily on the former but not on the latter. In liquid cultures of autoclaved aqueous leachings from the same type of soil, the fungus made abundant growth in accordance with the amount of sugar added. Menon concluded that the normal suppression of growth of *S. rolfsii* on Houston black clay is related not to the high calcium content of the soil but to an abundance of antagonists among the microflora of that soil type.

Mohr (15) studied possible calcium effects on the anatomy of the host as an explanation of the control that sometimes follows applications of calcium nitrate fertilizer. In tomato, a species highly susceptible to southern blight, Mohr observed variations in the extent to which the protective sheath of periderm is formed near the base of the stem in older plants. In ordinary commercial types, the development was slow and the sheath was not continuous. The application of calcium nitrate to young plants resulted in a marked hastening of the development of periderm, and that produced was more nearly continuous. The effect appeared to be related to the intake of calcium in the readily available form of calcium nitrate, since no such anatomical acceleration took place after applications of noncalcareous nitrogen fertilizers. The effect described appeared to be at least partially subject to genetic control, since plants of the T-514 strain of *Lycopersicon pimpinellifolium* exhibited this type of anatomical resistance to a higher degree than did standard tomato varieties.

In a factorial study of the effects of nitrogen, phosphorus, and potassium on injuries induced by *S. rolfsii* on peanuts in greenhouse sand cultures, only nitrogen was associated with any measurable effect (8). High nitrogen was related significantly to reduced losses from southern blight. Hudgins interpreted the effect as primarily on the ability of the host to recover from lesions initiated early in the life of the plant.

It has been a fairly common observation that losses from southern blight seem to be much more severe when wet weather follows a protracted dry period than when moisture is evenly abundant. One of the best documented examples was reported in 1952 for commercial carnations in central Italy (1). A drought of that year was almost unbroken from May 5 to late July, with maximum temperatures frequently above 35°C, and reaching 39°C during the first 10 days of July. On July 27 to 29, 86 mm of rain fell. The next rain (144 mm) came on the night of August 20. During the next few days maximum temperatures reached 30°C and the dying of carnation plants reached the proportions of a severe epidemic. The causes of a pattern of disease occurrence of this type have not been identified, but drought conditions might enhance the vigor of sclerotial germination after resumption of favorable moisture and temperature. Such was reported by Watkins (19), who found that mature sclerotia produced on autoclaved oat seeds germinated poorly. The percentage of sclerotia germinating and the vigor of germination were greatly increased by storage of the sclerotia at very low relative humidity levels for a few days. Comparable batches held at high atmospheric humidity continued to germinate poorly for several months. In this work humidity was controlled with various concentrations of sulfuric acid in glass jars. Boswell (2) used a similar technique, and also dried sclerotia by various exposures to warm air. He concluded that the dormancy of sclerotia of *S. rolfsii* is related to the continuity of the rind of those bodies. An intact rind was associated with slow, depauperate germination. According to Boswell, the sclerotia became wrinkled during drying, and the surface layers showed considerable cracking. He found that sclerotial dormancy could be broken by any of several types of mechanical or chemical scarification.

The growth of *S. rolfsii* in nature is almost always prominent at or near the soil surface. Host organs are most commonly attacked by the mycelium in that zone, which engenders some speculation that this fungus has higher demands for oxygen than do most soil-borne microorganisms. This tendency was noted by Taubenhaus (18), who suggested that the disease could be controlled if the mycelium and sclerotia were buried more than 5 in. deep. Flados (6) performed a number of experiments designed to determine the relation between oxygen level, growth of the fungus, and role of other soil microorganisms. In a series of oxygen-nitrogen mixtures ranging from pure oxygen to pure

nitrogen, *S. rolfsii* grew well in all atmospheres containing any oxygen at all. In certain cultures deliberately exposed to contamination from the air, the contaminating molds showed a relative growth advantage in atmospheres with oxygen at 5% or below, suppressing mycelia of *S. rolfsii*.

In tubes packed with soils and inoculated with *S. rolfsii* at the surface, Flados (6) found that the fungus remained near the surface in both soils used (Lakeland loamy sand and Bienville fine sandy loam) unless the soils were autoclaved. Then the mycelium of *S. rolfsii* grew to a depth of several inches.

From data presented by Flados it appears that the conspicuous concentration of the growth and pathogenic activities of *S. rolfsii* near the surface of the soil is not related primarily to the concentration of oxygen but to the distribution of antagonists among the soil microflora.

A number of writers have been concerned about the mechanics of the infection process as carried on by *S. rolfsii*. Taubenhaus (18) described the accumulation of masses of hyphae on the surface of the host, and the death of host cells near these masses. He commented on the "distinct zone of demarcation preceding the rotted area," and noted that this zone did not show hyphae. Taubenhaus thus noted the principally chemical invasion that exemplifies the "action in advance," first described by de Bary (3, v. pp. 410-411) for *Sclerotinia* sp. on stems of *Vicia faba* and found by many subsequent workers to be typical of the parasitism of most facultative parasites. Descriptions of the process by which *S. rolfsii* invades host tissues also appear in the accounts of Edson and Shapovalov (4), Higgins (7), and Paintin (16). There is reasonably close agreement on the microscopic details of the process of infection. Edson and Shapovalov made crude enzymic extracts from the mycelium and demonstrated the ability of such preparations to macerate tissues of potato tuber. Positive results from this kind of experiment have long been accepted as evidence of pectinase activity. Without direct experimental evidence Taubenhaus and Paintin similarly interpreted the parasitic action of the fungus to be primarily by enzymes, because of the characteristic details of cellular degradation shown in stained sections through lesions.

Higgins emphasized the importance of the oxalic acid produced by the mycelium as the primary factor in the killing of host tissues. The present reviewer does not consider either enzymes or oxalic acid to be exclusive agents in this connection. In a fairly similar example it was shown for *Phymatotrichum omnivorum* on cotton that the hyphal secretions contained both thermolabile and thermostable substances, and that certain definable aspects of the microscopic picture of "action in advance" could be attributed to substances in each category (20).

In *S. rolfsii* we have an environmentally somewhat unfastidious species that acts pathogenically as a facultative parasite, according to the classical concept of de Bary. It can grow on the kind of simple array of inorganic salts likely to be found in most soils of fairly low fertility. It does require an external source of thiamine, or at least of the intermediate, pyrimidine, which in nature would seem to limit the organism to soils containing at least fair amounts of plant remains or a reasonably abundant microflora. On the other hand, the soil microfloras tend to contain antagonists that may limit growth, and there is much evidence of the exceptional importance of this factor with *S. rolfsii*. Having at its disposal a large complement of exogenous carbohydrases, it can use as carbon sources a wide assortment of sugars, polysaccharides, and gums. These matters seem to be related directly to the broad range of hosts recorded for the fungus. The two indicated categories of invasive substances produced by *S. rolfsii*, oxalic acid and carbohydrases, are water-soluble, and thus readily absorbed by most host cells but not by the suberized cells of a periderm. Thus far, sheaths of periderm constitute the only anatomical basis on which to account for resistance.

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STRAINS OF, RESISTANCE TO, AND ANTAGONISTS OF SCLEROTIUM ROLFSII

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The widespread distribution of *Sclerotium rolfsii* Sacc. throughout the tropic and warmer portions of the temperate zones and its extensive and varied host range have stimulated inquiry into several interesting problems, including: 1) the development of morphological, physiological, and pathogenic strains, especially with geographical isolation; 2) the occurrence and nature of resistance to such an omnipathogenic organism; and 3) the influence of antibiotic and parasitic microorganisms upon the pathogen. This is a brief review of the literature and current work in these areas.

Strains.—Several investigators (2, 21, 38, 43, 44) have compared the morphology and host range of *S. rolfsii* isolates from various hosts, and concluded that the differences in the cultures studied were relatively small. Nakata (36) demonstrated the stability of morphologic characteristics by repeatedly subculturing 49 isolates and observing only 2 mutants after 2 years of frequent transfers (37). Epps et al (9) and Lyle (25) confirmed the extremely low rate of mutation. Although Welch (46) described *S. delphinii* Welch as having larger sclerotia than *S. rolfsii*, Stevens (41) concluded that these species were closely related but that differences may warrant retention of *S. delphinii* as a separate species. Lyle (25) concluded that *S. delphinii* fell within the limits of the species *S. rolfsii* as indicated by variation of single-basidiospore cultures from a parental culture of the latter species.

From a detailed morphological comparison, Goto (15) classified 33 Formosan and 7 foreign strains into 4 types: I) readily sporulating; II) sporulating; III) rarely sporulating; and IV) nonsporulating. In general, the sporulating types (I and II) had more fluffy aerial mycelium and more numerous smaller sclerotia than the rarely and nonsporulating types. Further studies (16), including 30 foreign strains of *S. rolfsii* and *S. delphinii*, indicated that the American strains were usually of types I and II, which he considered typical *S. rolfsii*, whereas the common Indian strains and *S. delphinii* were of type IV.

Single-basidiospore cultures from 3 sporulating cultures resulted in a high frequency of aberrant forms from each culture (17). Pairing these aberrant single-basidiospore cultures frequently gave typical *S. rolfsii* cultures. The variability between single-basidiospore cultures and the recovery of the wild type by these pairings (production of the dicaryon) was interpreted by Goto (17) as indicating heterothallism. The variability in morphological characteristics of single-basidiospore cultures from a single hymenium was later confirmed by West (47) and Lyle (25). Mundkur (33) and Lyle (25), however, found that some single-basidiospore cultures produced a hymenium and mature basidiospores, indicating homothallism.

Curzi (5) attempted to correlate the morphological and physiological characteristics of the sclerotial stage with the characteristics of the basidial stage. He concluded that there are several species confused in the form species *Sclerotium rolfsii*. He described *Corticium rolfsii* (n. comb.) as the perfect stage of the common type, *S. rolfsii*. This was distinguished from *Corticium centrifugum* (Sev.) Curzi in both the sclerotial and perfect stages (6).

The aversion phenomenon between cultures of *S. rolfsii* was reported first by Taubenhaus (43), who considered it a sexual response. In an extensive study Nakata (34) found 37 aversion strains in the 47 cultures studied, and associated these with morphological differences. He later concluded that aversion was due to substances diffusing from the mycelium into the medium (35). Among the other types of physiological variation, Higgins (21) and Nakata (36) reported strain differences in growth response to pH levels. Lyle (25) found a basidiospore isolate that required thiamine for sclerotial production. Abeygunawardena and Wood (1) demonstrated strain variations in lactose utilization.

Several workers (2, 43, 44) failed to find differences in pathogenicity of isolates from various hosts. West (47) found no differences in pathogenicity of morphologically variable basidiospore cultures from a basidial mat. Edson and Shapovalov (8) reported 1 of 2 isolates to be slightly more virulent in potato seed-piece decay. Epps et al (9), comparing the pathogenicity of 4 isolates on several hosts, demonstrated differences in rates but no difference in total number of plants killed.

Over a 2-year period Cooper (3), using mass inoculation techniques, compared 52 isolates on field-grown peanuts. Very weakly to highly virulent isolates were found, and correlation for isolate scores between tests was good. In subsequent studies, isolates selected as weakly virulent remained so whereas 2 of 5 virulent cultures were less virulent. This indicates that a loss of virulence in culture may account for some of the observed differences. More recently, how-

ever, in greenhouse tests, 10 recently collected isolates gave disease indices ranging from 20 to 79% (Cooper, unpublished). Although some weakly virulent isolates were from peanuts, all isolates from other species in this test were weakly virulent on peanuts.

In brief summary of the strain situation, extreme morphological and less marked physiological differences are known. Isolates from a given geographic area may be relatively uniform, and demonstrable mutants are very infrequent. Even so, single-basidiospore cultures from one of these isolates frequently show the extreme variation of the species. Although homothallism has been demonstrated in some cultures, this extreme variation between single-basidiospore cultures indicates heterothallism. The uniformity of isolates from an area may be an expression of dominance in the dicaryon.

Differences in isolate virulence have been shown by using tolerant to resistant hosts as test plants. Host specialization, however, remains to be conclusively demonstrated.

Resistance.—The pathogenicity of *S. rolfsii* with an extensive host range of over 189 species (44) ranging from mosses to composites has been attributed to the exogenous production of oxalic acid, a nonspecific metabolite (21), and more recently to the production of a wide array of enzymes for carbohydrate metabolism (23). The above generalizations might indicate a low probability of obtaining resistance within a susceptible species. Even before the pathogen was named, however, Fulton (13) had demonstrated varietal resistance in pepper.

Soon thereafter McClintock (27, 28) reported that Virginia Runner peanuts were more resistant than other varieties. Reyes (38), using precise inoculation techniques to compare the resistance of 13 varieties for 3 successive years, found a high correlation ($r = .712$) between percentage of plants killed and yield reduction for each variety (Table 1), and clearly demonstrated the range of susceptibility. Runners were generally more resistant than decumbent and erect types.

Table 1.—Summary of infection and yield reduction for peanut varieties inoculated with *S. rolfsii* (1934, 1935, 1936).[a]

Variety	Type	Stem rot (%)	Yield reduction (%)
Valencia	Semi-runner	59	45
Macapno	Decumbent	57	34
Georgia Red	Decumbent	46	50
White Improved Spanish	Decumbent	44	25
Spanish	Decumbent	44	5
Biit	Decumbent	42	42
Cagayan #1	Decumbent	42	26
San Jose #3	Decumbent	39	24
Vigan Lupog	Semi-erect	19	19
Tirik	Semi-erect	19	24
Tia-Tau[b]	Runner	8	3
Virginia Jumbo	Runner	4	10
Virginia Jumbo (a)	Runner	3	9

[a] Adapted from Reyes (38).
[b] Not included in the 1934 test.

In 1952, 718 entries in a plant introduction nursery in a naturally infested field were scored before harvest for obviously infected plants and at harvest for sublethal infections and pod rot. Range in susceptibility was extensive. The following year 19 apparently resistant, 3 susceptible, and 3 varietal entries were compared in replicated inoculated and uninoculated plots. The range in percentage of stems killed, 8–93%, and in yield reduction, 14–74% generally confirmed the previous classifications (Cooper and Gregory, unpublished). Discrepancies, however, indicate the inadequacy of natural infestation and a single replicate in evaluating disease resistance.

A study of *S. rolfsii* resistance in varieties and advanced breeding lines indicates a narrow but highly significant resistance-susceptibility range in the Virginia type peanuts. The varietal data (Table 2) show the relative susceptibility of varieties currently grown in the Virginia-Carolina area (Cooper and Gregory, unpublished). Garren (14) found no difference in susceptibility between Virginia Bunch and Virginia Runner types.

Table 2.—Comparative susceptibility of peanut varieties grown in the Virginia-Carolina area to *S. rolfsii* (1958–59).

Variety	Pod rot score[1] (1958)	Stems killed (%) 1958	1959
Va-56R	4.00	54.8	89.3
Ga-119-20	4.00	24.2	86.1
NC-1	3.75	35.8	86.0
NC-4X	3.50	38.0	85.3
NC-4	3.50	34.5	74.4
NC-2	2.00	11.0	67.8
LSD .05	0.842	15.75	15.10
.01	1.114	20.84	19.99

[1] 0 = no pod rot; 1 = 1–10%; 2 = 11–25%; 3 = 26–50%; and 4 = 51–100% pod rot.

Resistance to *S. rolfsii* has also been reported for other crops. Twenty-one of 126 rice varieties escaped infection in a Philippine nursery (30). Texsel, an early maturing upright guar variety, was resistant (42). Resistant strains of common blue lupine were isolated (7). Preliminary studies indicate resistance in certain Delphinium varieties (29). A strain of *Lycopersicon pimpinellifolium* Mill. was highly resistant at temperatures below 30°C. This resistance was inherited as a monofactorial (31) dominant. Radiation of Virginia Bunch peanut seed with X rays induced variants both with increased susceptibility and, less frequently, with increased resistance (4).

The nature of resistance is not clearly defined. Higgins (21) attributed the resistance of peanut stems to a thick cuticle with few stomata. The resistance in the *L. pimpinellifolium* strain was associated with thick-walled cortical cells and cork cambium activity in invaded tissues (31). Several workers have noted that the resistance of some plants increased with age (10, 21), and Epps et al (9) concluded that plants with woody stems and roots generally become more resistant with age (cotton, cowpea, soybean, and

tomato) whereas plants with herbaceous and fleshy roots remained susceptible (beans, sugar beets, and carrots). Some herbaceous plants, however, were resistant throughout their lives (garden peas). *Hibiscus sabdariffa* L. var. Victor is reported to become more susceptible as it reaches maturity (39). To the author, however, this appears to be the result of a more favorable environment, dense shading with high humidity, rather than a change in susceptibility.

In summation, varietal resistance to this almost omnipathogenic organism has been demonstrated in a number of hosts. This resistance has been attributed to an impervious cuticle, thick-walled cortical cells, and cork cambium activity or merely klendusic activity. Inheritance of resistance in *L. pimpinellifolium* is a monofactorial dominant. In other species it is multifactorial or unknown. Although varietal immunity has not been shown conclusively in susceptible species, the above examples indicate a possibility of the discovery of greater resistance in at least tolerant to moderately resistant species.

Antibiosis.—*S. rolfsii* is capable of extensive saprophytic development with adequate undecayed plant debris and suitable physical environment, although it has been found to be subject to certain biotic factors. Weindling (45) demonstrated that *Trichoderma lignorum* (Tode) Harz parasitized as well as inhibited the development of *S. rolfsii* mycelium, and preliminary experiments indicated a degree of biological control.

Ferguson (11) found that mechanical injury, leaching, or active respiration of live *S. rolfsii* sclerotia did not appreciably increase colonization by other soil organisms. Sclerotia killed by either chemical or physical means, however, were equally susceptible to colonization. Only *Trichoderma* or *Penicillium* spp. were isolated from intact or wounded sclerotia, whereas bacteria, fungi, protozoa, mites, and nematodes were associated with dead sclerotia.

In a survey for antagonistic activity in the microorganisms isolated from Louisiana soils, Morton and Stroube (32) found that only 0.2%, 1.7%, and 3.5% of bacteria, actinomycetes, and fungi, respectively, were antagonistic to *S. rolfsii* in culture. These antagonistic actinomycetes and fungi generally reduced disease severity in greenhouse tests in reinfested soil. The antagonistic bacteria were not effective in reducing disease severity.

Antagonism has been suggested as a factor in the following cases: 1) survival of *S. rolfsii* sclerotia following soil fumigation (26); 2) disease severity following the addition of nitrogenous materials (22, 24) or soil fumigation (18, 19); and 3) rate of mycelial development in nonsterile soils (12).

The frequency of isolation and antagonistic nature of *Trichoderma* sp. and *Geotrichum* sp. isolated from tissue of white clover infected with *S. rolfsii* indicate the possible role of these organisms on disease development (20).

In contrast to antagonism, certain fungi grown with *S. rolfsii* stimulate its mycelial growth and/or sclerotial formation. On an alkaline medium, *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hans. reduced pH to levels favorable for *S. rolfsii* development (40). On a thiamine-deficient medium, 81%, 68%, and 66% of the bacteria, fungi, and actinomycetes, respectively, stimulated mycelial development (32). There was, however, no increase in disease severity by adding the stimulating organism to the soil with *S. rolfsii* inoculum.

In conclusion, *S. rolfsii* is relatively resistant to antagonism or parasitism by the soil microflora. This may contribute to its persistence in the soil and thus to its resistance to control by practices such as crop rotation.

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COMMENTS ON PART I: SOME GAPS IN OUR KNOWLEDGE OF SCLEROTIUM ROLFSII

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The papers presented here show that *Sclerotium rolfsii* is a well-known fungus with some well-kept secrets.

The first of these secrets concerns the variation in morphological characteristics. An indefinite number of types can be collected, showing a wide range in sclerotial characteristics ranging from those typical of *S. delphinii* to those typical of *S. rolfsii*. Yet, within a given locality, the types collected tend to be remarkably uniform. How do these morphological variations come into existence? In culture, the fungus sectors, or mutates, rarely; and we might assume that the same is true in nature. My personal experience with sectors, or mutants, has led me to conclude that the most common type of morphological change is the formation of nonsclerotial strains. This type would not be expected to survive in nature.

The most obvious explanation is that the various types arise from basidiospores. In the laboratory a number of different types of cultures may be obtained from single basidiospores from the same basidiospore-producing culture. This proves that basidiospores can give rise to new types. Two objections can be raised to this explanation, however. In the first place, the perfect stage is not regularly produced in culture, and has been observed very infrequently in the field. In the second place, cultures from single basidiospores vary widely. Thus, it might be expected that, if basidiospores give rise to new types in nature, there would be many different types in a given locality. As mentioned earlier, that is not true. Dr. Cooper pointed out that the uniformity of isolates in a given area may be an expression of dominance in the dicaryon. Even

if so, it does not explain the variation in types found in different geographical areas.

A second secret of this fungus is closely related to the first. As far as I know, no one has been able to establish the pattern of nuclear behavior during the spore forming process. Many interesting questions can be asked. Is a true diploid nucleus formed that later undergoes reductional division? Are the basidiospores and germ tubes uninucleate? If so, how and when is the binucleate, or multinucleate, condition established? Is the fungus homothallic or heterothallic, or are some cultures one and some the other? That would appear to furnish a fruitful field for basic research.

From the practical standpoint, the most important gaps in our knowledge of *S. rolfsii* are those related to the pathogenic activities of the fungus. Taubenhaus, Higgins, Paintin, and Edson and Shapovalov have made significant contributions to our knowledge of the mechanism of action during the initial phases of infection and subsequent destruction of tissue. Our knowledge of pathogenesis, however, is far from complete. Does true resistance actually exist within certain species, or are we dealing with the old problem of "disease escapes?" The so-called resistant strains of certain varieties can be destroyed by *S. rolfsii* if conditions are made favorable enough for the fungus. These plants remain healthy when exposed to the fungus under less trying conditions, such as those that prevail in field plantings. Our understanding of the nature of resistance in the host is dependent on a better understanding of the nature of parasitism by the fungus.

Parasitism is associated with vegetative growth, which is influenced by a multitude of factors. Temperature and oxygen requirements have been established, but in the area of "association-effects" of other microorganisms we have hardly scratched the surface. It has been demonstrated that certain microorganisms suppress, and certain organisms stimulate, vegetative growth. How important are these effects in the field, where other factors such as pH, base exchange capacity, and temperature operate? The work of Flados raises some interesting questions. If the fungus grows best on or near the surface of the soil because of the absence of antagonistic microorganisms, it should be possible to achieve control by promoting the growth of these organisms on the surface of the soil. Such a concept is contrary to generally accepted theories.

Parasitism, or pathogenicity, cannot be separated completely from ecology, but fundamental research on the identity of the enzymes produced and the factors affecting this production, and on the chemistry of the substances that destroy host tissues, would answer some very important questions.

THE ECOLOGY OF SCLEROTIUM ROLFSII WITH EMPHASIS ON THE ROLE OF SAPROPHYTIC MEDIA

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Ecology has been compared to an object encased in a porous sphere. What you see depends on the type of scope you use and which pore you look through (2). The semantic and pragmatic problems involved in recording and interpreting such multiple observations are often difficult. Even the concept of *environment*, or of ecology itself, may become hypothetical and confused (3). To unravel some of these problems in an exhaustive bibliographic review of *Sclerotium rolfsii* Sacc. is not the intent at this time. With the purpose of disease control in mind, let us sift out and recount some of these more pertinent and consistent ecological observations. This may appear much a matter of hypotheses and words, but the results when these hypotheses are put into "actions that speak louder than words" will be discussed by the next speaker.

From an ecological point of view, the basic observations and data necessary for a practical control of southern blight were reported by Taubenhaus (4) and Higgins (1).

In outline, the organism has two phases that are ecologically distinct. First, there is the mycelial development that forms the heavy white growth from which the fungus gains the common name, "white mold." This might also be referred to as the growth phase, or pathogenic phase, of the fungus. Second, there is the less spectacular production of sclerotia, which enables the organism to survive adverse periods. The environment will also be divided into what may be called the inorganic and organic phases.

Let us first consider the inorganic phase of the environment. A combination of factors that results in a warm, moist, well aerated condition on or in the upper inch or so of soil appears to favor the mycelial development of this fungus and increase its severity as a pathogen. This condition develops during periods when weather records show that temperatures have been 85–95°F during most of the day and seldom dropped below about 75°F during the night. Showers are also essential to maintain a moist, but not excessively wet, condition at or near the soil surface. The fungus also appears to be distinctly aerobic as far as its mycelial, or pathogenic, development is concerned.

There appears to be good agreement in the literature on the range of temperature, moisture, and aeration most favorable to the mycelial or pathogenic phase of this organism. Some workers, however, have tried to explain differences in the distribution and severity of this disease on the basis of the aerobic factor in the soil. Such explanations are generally based on short-term surveys, or comparisons between one or two specific locations. The factor of aeration appears to be more of a coincidence than a causal relation that could be taken as a basis for a general recommendation for control. In my own experience with peanuts in Georgia, I have watched this disease cut with almost razorlike precision at the surface of heavy soils, whereas in lighter soils a greater depth on the stem or root may be involved. But from the practical standpoint of control, the result was the same.

The pH, though not necessarily a strictly inorganic factor, is considered under this category. This factor does not appear to be a critical one from the standpoint of practical control of the fungus. The limits of pH reported to affect the organism significantly are well beyond those for practical production of most crops.

Inorganic salts, at least in the amounts found in cultivated soil, appear to have no significant effect on the development or pathogenicity of this organism. It has been reported that certain elements of fertilizer affect the susceptibility of the peanut because of the prevalence of disease in a field-plot test. Hypothetically speaking, if this test were made in a soil deficient in the particular element under study, its use would increase the production of the peanut plant, and most likely the severity of leafspot. The leaves that would shed as a result of the leafspot could increase the chances of stem blight or peg rot due to *S. rolfsii*. That is just one example of the many that have made the peanut the "unpredictable legume." This combination of host and parasite is one that cannot be considered in a piecemeal way if our goal is practical control.

In summary, as far as the pathogenic phase of the fungus is concerned, the inorganic phase of the environment is primarily meteorological. The more critical factors are in the weather, which we cannot control or avoid with crops that are susceptible through their entire growing season of 4–5 months.

As we turn from the inorganic to the organic phase of the environment for this fungus, two factors appear most pertinent: 1) the sources of energy, or food available to the fungus, and 2) the character of other organisms in the environment.

The importance of available food, or source of energy in the development of a control for this fungus may be outlined as follows. Let us start with a sclerotium that has survived conditions adverse to active growth of the fungus.

1) This sclerotium does not contain sufficient energy within itself to establish a parasitic relation to a susceptible host, even though inorganic factors may be favorable. 2) If we supply the sclerotium with a source of energy such as a necrotrophic or saprophytic medium in this favorable environment, growth of mycelium is rapid. Under such conditions, this mycelium is capable of producing the quantity or concentration of metabolites required to kill a spot in the live tissue of a proximate host. 3) After this has happened, the fungus penetrates the dead spot and we have a fungus taking lodgement and subsistence in the dead tissues of an otherwise live host plant. Since the by-products of this fungus growth are necrogenic, the result has been described as parasitic or expensive to the host.

The first point above, regarding whether or not the sclerotium contains enough energy to establish a parasitic relation with its host is as yet somewhat hypothetical. There are records of infections obtained by placing a sclerotium next to seedlings or plants growing under artificial conditions in glass vials or greenhouse culture. However, under these conditions the possibilities of available nutrients such as might diffuse from a seed germinating on an agar medium, or from dead algae, necrotic roots, etc. in the soil have been ignored.

The question regarding the role of saprophytic media in infection is further illustrated by an observation reported in an early classical study of this problem (1). "In soil containing large quantities of undecayed organic matter, every plant may be killed; yet susceptible plants have been grown free from attack over long periods in soil containing numerous sclerotia of the fungus." Hypothetically, the reason why such plants were not infected was that the sclerotia had no undecayed organic matter to use as a source of energy in their attack of the plant. This hypothesis has been basic in the development of a very practical and successful control of southern blight on peanuts.

This phenomenon is well described by the words necrotrophic parasite or pathogen. The word *necrotrophic* means, in a literal sense, dead food. To recount, we have an organism that survives by means of sclerotia. These sclerotia do not have the inherent energy to produce metabolites in a necrogenic concentration without what has been variously called a food base, a necrotrophic or saprophytic source of energy. The next speaker will describe what occurs when such sources of energy are moved by proper tillage from the most favorable to a less favorable ecological stratum in the soil and how this tillage must be followed by cultural practices that prevent any accumulation of necrotrophic media in this favorable level of soil while the crop is growing.

So far in our discussion, reference has been limited to the mycelial, or pathogenic, phase of the fungus. Ecological conditions that check the development of this phase, favor the production of sclerotia. These sclerotia not only enable the species to survive but materially increase the inoculum and chances of infection when circumstances are again favorable.

Available food has a great effect on which phase of growth predominates with this fungus. Oat seeds that

have been soaked and autoclaved are often used as a laboratory culture medium. When seeds of high test weight are used, the growth will be predominantly mycelial, with very few sclerotia, if any. In contrast, if the oats are of extremely low test weight, as the waste from a seed cleaner, the mycelial growth will be relatively sparse and sclerotia will develop in great numbers. Specimens such as pieces of corn stalks or wads of organic trash reclaimed from the cooler (somewhat anaerobic) depths of a field are often sparsely covered with mycelium but well covered with sclerotia. It is evident from such specimens that the susceptibility of a live host has little relation to its value as a medium for the production of sclerotia. For this reason, there appears little opportunity to reduce the amount of inoculum by rotation based on the resistance or susceptibility of live plants, as with biotrophic pathogens.

Where control of a disease due to a soil-inhabiting fungus is obtained without the use of chemicals, or consideration of the susceptibility of rotated crops, questions regarding the possibility of antibiotic or antagonistic flora appear frequently. There is ample evidence that many soils do contain flora having such a potential, but for the following reasons these do not appear to be a primary factor in the control of this organism on peanuts. Plants growing in rows where the upper 3–4 in. of soil is void of undecayed organic matter are free of infection, whereas in adjacent rows with organic matter in the upper inches of soil, infections may severely reduce stands. If the soil is ridged up to cover the lower nodes on the healthy plants, one or more stems on 85% of the plants may soon be infected. This is a very common occurrence and the infections are found where leaflets have been smothered against the stem. These smothered leaflets are the necrotrophic or saprophytic medium required by *S. rolfsii* to create pathogenesis and eventual parasitism. In such cases, we must conclude that antagonistic flora are either too rare or too slow in action to be of practical significance. Below depths of 3–4 inches in the soil, ecological factors such as temperature and aeration check the pathogenic growth of the fungus. At this level in the soil, antibiotic or antagonistic flora would be a matter of coincidence rather than a primary factor in control of pathogenesis. Theoretically such phenomena could reduce the number of sclerotia formed at this level in the soil. So far this factor has appeared of no significance in the development of practical control methods.

When our control practices are described under so many different specialty names, it is easy to overlook the basic significance of such a common everyday term as "food." Fundamentally our breeding of resistant or immune varieties of plants is a matter of food control. We breed to change some molecule in the plant that will render it an unsuitable source of energy for an obligate or biotrophic parasite. Or in other cases, we find we can breed plants with certain morphological or histological characters that form a barrier between the parasite and its food. We follow certain practices such as dates of planting, rotations, etc. to avoid the ecological circumstance under which the plant can serve as a source of energy to the parasite.

This principle of food control can be extended to apply with fungi of a more saprophytic or necrotrophic type, such as *S. rolfsii*. We can control the food of such a fungus by first following methods of tillage that bury all cover crops or crop refuse and weeds in an ecologically unfavorable stratum in the soil. This must then be followed by cultural practices that avoid bruising, insect damage, smothering, leaf shed, transplanting or any other thing that would create any dead or morbid tissue proximate to the plant and in the more favorable upper stratum of soil. In this way the fungus is denied a source of energy in the ecological stratum of soil most favorable for pathogenesis.

Failure to distinguish between the biotrophic and necrotrophic character of a pathogenic organism can lead to some very confusing results. The peanut crop is a good example of this. Failure to recognize the necrotrophic character of this fungus which is so common on peanuts, contributed much to the confusion that resulted in the peanut becoming known as "the unpredictable legume."

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CONTROL OF SCLEROTIUM ROLFSII THROUGH CULTURAL PRACTICES

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Control of *Sclerotium rolfsii* Sacc., a problem with many crop plants, has been attempted by fertilizer and other chemical treatment (discussed elsewhere), rotation, and tillage. Other procedures have also been investigated. For example, in 1921 Tisdale (15) suggested changes in time of planting as a control measure, because he found that rice sown later than usual grows vigorously and escapes much infection by *S. rolfsii*. These miscellaneous procedures have not been widely accepted, however, and the main cultural control practices are rotation and tillage procedures. Their effects on the infection of plants by *S. rolfsii* are discussed herein.

Much of the published material on the effect of rotation and tillage on *S. rolfsii* is concerned with the stem rot of peanuts (*Arachis hypogaea* L.) (8). The multiple stems arising near or slightly below the soil surface make peanuts unusually susceptible to *S. rolfsii*. The underground peanut fruit is attached by stemlike stalks called "pegs," which are highly susceptible to *S. rolfsii*. The widely used "ridge cultivation" of peanuts actually increases the amount of stem rot. This is primarily a report on cultural practices and control of *S. rolfsii* on peanuts.

Rotation.—*S. rolfsii* has a wide host range. Apparently almost all broad-leaved weeds found in the southern United States are susceptible as are most of the major crop plants of this area. McClintock (9) noted the longevity of *S. rolfsii* in 1917, and cautioned against placing much hope in rotation as a means of controlling it on peanuts. Nevertheless, for several years after, control of *S. rolfsii* was thought of mainly in terms of rotation. Rotation is now generally recommended for control of *S. rolfsii*, but in few instances is the recommendation based on published data from rotation studies.

In 1956 Cooper (7) reported preliminary results of a long-range rotation study with peanuts. Recently, he furnished additional data from the study. Over a 7-year period (1952–58) average infection by *S. rolfsii* was 9% for peanuts grown every year; 5% for peanuts alternating with soybeans; 3% for peanuts alternating with corn; 2% for 3-year rotation of wheat, lespedeza, and peanuts; and 2% for 4-year rotation of wheat, lespedeza, corn, and peanuts. The infection levels seem low, but the trends indicated are in line with unpublished observations of other investigators and experiences of growers.

The writer conducted an experiment for the past 5 seasons on the effects of certain cultural practices on stem rot of peanuts incited by *S. rolfsii*. For 3 of these seasons the experiment was located on a severely infested field. In 1956 the average of infected plants was 18.5%. When the test was repeated, plot for plot without rotation, in 1957, the incidence of infection by *S. rolfsii* increased to 35.8%, but after 1 year of a crop of lower susceptibility the infection was 24.8%.

Tillage procedures.—Some suggestions for possible control of *S. rolfsii* through tillage procedures are aimed at the sclerotia. Tisdale (15) suggested in 1921 that early plowing followed by thorough disking would promote germination and exhaustion of sclerotia. In 1940 Bottomley (2), in South Africa, suggested stirring the soil immediately after harvesting peanuts to promote drying out of immature sclerotia. She also recommended sanitation measures to remove sclerotia from infested fields.

But the majority of the tillage procedures recommended for control of *S. rolfsii* are concerned with the destructive phase of the fungus, and these recommendations attest to a general recognition of the importance of organic matter in the ecology of *S. rolfsii* and that *S. rolfsii* is active mainly at or near the soil surface. By analysis one may see that each of these tillage procedures is suggested in the hope that it will have one or another of the following effects: a) limit the oxygen supply of *S. rolfsii;* b) promote the decay of organic matter; c) remove organic matter from the infection zone; and d) prevent introduction of new organic matter.

Controlling oxygen supply of S. rolfsii.—In 1919 Taubenhaus (14) discussed the role of aeration in infection by *S. rolfsii*, and suggested deep plowing to bury the fungus. In 1940 Bottomley (2) recommended deep plowing to bury mature sclerotia. Controlled flooding can also achieve the end of depriving *S. rolfsii* of oxygen. Tisdale (15) noted that when rice seed beds are dry the disease caused by *S. rolfsii* is at its worst. Therefore he recommended keeping seed beds flooded. Flooding is suggested as a control measure for *S. rolfsii* on various crop plants in India (16). Controlled flooding must be practiced with extreme care, however, for excessive moisture is reported to favor infection by *S. rolfsii*. Mejia (10) recommended sowing the seeds of *Sindora supa* Merr., a Philippine tree, during the drier part of the year, and avoiding heavy watering to escape severe infection by *S. rolfsii*. Both Clinton (6) and Bottomley (2), working in Africa, noted that *S. rolfsii* is worse on peanuts in wet seasons, and particularly in spots subject to flooding. No similar observations have been recorded in the United States.

Promoting decay of organic matter.—In 1921 Tisdale (15) noted that *S. rolfsii* is such a vigorous saprophyte that the organic matter in the soil and the organic debris on the surface should be considered when management practices are developed for areas with a high degree of *S. rolfsii* infestation. He recommended early plowing of infested rice fields, followed in a few weeks by thorough disking to promote decay of organic matter. In 1947 Sellschop (12), in South

Africa, recommended that the trash of every crop plant grown immediately before peanuts be plowed in directly after harvest to prevent multiplication of *S. rolfsii* on the trash.

Removing organic matter from infection zone.—A somewhat different solution for the organic matter problem—tillage to remove the organic matter from the infection zone by burying it below the infection zone—has found considerable favor. By 1937 "clean cultivation" had become a standard recommendation for control of *S. rolfsii* on peppers (1). The burial of organic matter by careful plowing for controlling *S. rolfsii* on peanuts was first recommended by Boyle (3), in Georgia in 1952. This practice is now recommended for peanuts in North Carolina (11) and Virginia (8) as well.

Preventing introduction of new organic matter.—Organic matter can be introduced into the infection zone of *S. rolfsii* by two principal means: 1) defoliation of suscepts by foliar diseases, and 2) the cultivation procedure of "dirting," or piling soil into the rows of a crop plant to smother weeds or grass therein. Sometimes this procedure is practiced merely to "hill-up" the crop plant. "Dirting" brings leaves of the crop plant, weeds, and grass into contact with living stems of the crop plant. When such material dies from being covered with soil, it furnishes an almost perfect center for activity by *S. rolfsii*. In addition, the bruising, smothering, and shading of stems of crop plants by "dirting" creates spots of weakened or dead tissue within the stem. This seems to be a nearly ideal situation for the development of infection by *S. rolfsii*. In 1917 flat cultivation was reported as resulting in higher yield of peanuts than ridge cultivation (13), but it was not until 1949 that a relationship between ridge cultivation and development of *S. rolfsii* infection was noted. Ciccarone and Platone (5), working with several crops in Venezuela, first published in 1949 the concept that it is dangerous to cultivate so as to pile soil into the row because it allows *S. rolfsii* to come in contact with stems under conditions very favorable to the fungus. In 1952 Boyle (3) reported reduction in *S. rolfsii* infection on peanuts following use of pre-emergence herbicide and flat culture in Georgia, where these procedures were eventually recommended for control of *S. rolfsii* on peanuts (4). In 1952 Wells and Cooper (17), in North Carolina, noted that cultivating peanuts in late summer so that excessive hilling or bedding results usually increased the severity of stem rot. In 1955 (11) a North Carolina publication noted that severe outbreaks of stem rot of peanuts often result from hilling or bedding during late cultivations, and that these outbreaks are associated with the covering of green leaves and stems. Therefore flat cultivation was recommended for control of peanut stem rot in North Carolina. In Virginia the use of a pre-emergence herbicide and careful cultivation to avoid "dirting" was studied for 4 years, and these procedures are now standard recommendations for control of *S. rolfsii* in Virginia (8). Of course, everyone recognizes that control of foliar diseases on crop plants is an important part of control of *S. rolfsii*.

To avoid confusion, a word about terminology might be appropriate here. "Flat culture" as used by Boyle appears not to be the same as "flat cultivation" as used by the North Carolina investigators. To Boyle, "flat culture" means that a herbicide is used and no soil is thrown around or near the base of the plants during their entire development. Study of the publications cited from North Carolina (11, 17) suggests that "flat cultivation" does not involve use of a herbicide, that no more soil than necessary to control weeds is thrown around the base of the plants, and that hilling or bedding of peanuts during late cultivation is omitted.

Combination of procedures.—In 1952 Boyle (3) first reported results of an investigation of a particular combination: a) plowing for complete burial of organic litter on the surface of the soil, and b) control of weeds with a pre-emergence herbicide and flat culture. He felt that the results justified recommending these procedures for control of *S. rolfsii* on peanuts. In 1956 Boyle and Hammons (4) reported in greater detail on a field test of these procedures in Georgia. They compared two methods of land preparation—use of a moldboard plow to bury all weeds and trash more than 3 inches deep, and disking to mix trash and weeds into upper part of soil in combination with two methods of weed control—breaking soil onto the row and use of herbicide with no breaking of soil onto the row. Their published results and some further unpublished results of Boyle (included here by permission of Boyle) are given in Table 1. Boyle and Hammons attributed damage observed to stem and root rots caused by *S. rolfsii* and *Rhizoctonia solani* Kühn, and concluded that conventional methods of planting and cultivating not only favored the development of stem rot but also checked the normal development of Spanish peanut plants by suppressing lateral branching and reducing flowering.

From 4 years of study in Virginia, the writer (8) developed a similar combination of cultural practices for controlling peanut stem rot in Virginia. The Virginia plan is very simple, consisting of two procedures: a) *Deep covering* of any organic matter on the soil surface at the time of seed-bed preparation, and b) *non-dirting* cultivation to control weeds in the row without throwing soil around the base of the plants to smother the weeds.

Deep covering was achieved with a coulter and jointer mounted on an 18-inch-bottom moldboard plow. The trashy surface layer of the soil being turned was thrown by the jointer into the bottom of the last furrow plowed. The plow then buried this organic material under at least 4 inches of soil containing no fresh organic matter other than a few plant roots. Deep covering was achieved by careful use of the right equipment rather than by plowing to an unusual depth. Nondirting weed control was achieved by planting on a slightly raised bed and by using dinoseb (4,6-dinitro-*o*-secondary butylphenol) as a pre-emerg-

Table 1.—Relation of cultural practices to stem rot and Rhizoctonia root rot of Dixie Spanish variety peanuts in Georgia, 1956 and 1958 (data of L. W. Boyle).

Measure and year	Treatment			
	Moldboard		Disk	
	Herbicide	Cultivated	Herbicide	Cultivated
% of stand healthy				
1956	96.8	69.3	98.7	75.6
1958	97.3	92.2	98.1	89.7
Yield (lb/A.)				
1956	2188	2064	1851	1657
1958	2950	2080	2776	1903
% increase in yield				
1956	32	25	12	base
1958	55	9	46	base

ence herbicide in band treatments at the rate of 1.5 gal. of 53% active substance per acre. Only areas between rows were cultivated. Cultivation was by flat sweeps supplemented by dirt shields for the first cultivation. No soil was thrown around the base of plants during cultivation. Only infrequently was hand weeding necessary to control weeds and grass in the row.

The practices of deep-covering and nondirting were compared with opposites providing fairly broad contrasts. The opposite of deep covering, called "surface mulching," left cover crop and other surface litter in the upper few inches of soil. Surface mulching was accomplished by disking the seed bed thoroughly and then working it to a depth of about 8 in. with an all-purpose cultivator. The opposite of nondirting cultivation—"dirting"—approached conventional practices. Essentially it consisted of planting in a furrow and throwing enough soil into the peanut row with each cultivation to smother weeds as they developed.

One more year's data may be added to the previously published data (8), and the remainder of this discussion is based on these 5 years' data (1955–59). These 5 years' data (Fig. 1-A) substantiate the major conclusion, that tillage procedures can effectively reduce *S. rolfsii* infection on peanuts.

The infection data of Fig. 1-A were obtained by careful examination of a field of peanuts each week and tagging infected plants as soon as infection became apparent. For ready comparison the results are in graphic form in order of apparent infection level. The inverse correlation between infection and yield evident in Fig. 1-A was highly significant each year. The more severe was the disease, the higher was the negative correlation between infection and yield. Interaction between treatments had significant effects (5% level) on infection in 1956 and 1957, and on yield in 1957 and 1959. Throughout the study, the maximum infection was in the surface-mulched, dirted plots. Nondirting treatments were consistently the better ones in yield of pods as well as infection. The deep-covering, nondirting combination was the most effective for 4 years; and when the test was repeated without rotation, it was the only combination that did not show an increase in infection.

The amount of infection detected in the surface-mulched, dirted plots (Fig. 1-A) indicates that the infection level was low in the field used in 1955, intermediate in the field used in 1958, and high in the field used in 1956. When the test was repeated without rotation in this field in 1957, infection level was again high. An unusual combination of weather conditions, making for much premature shedding of leaves, may be largely responsible for the abnormally high infection in the nondirted plots in 1959.

In Fig. 1-B, since a correlation between infection and yield is established, each treatment is compared for infection only with its opposite, with the scale doubled. Data from the 1957 test are not used, since the test was repeated in 1957 without rotation and the intermediate infection level found in 1958 is classified in this graph as a low infection level. Fig. 1-B shows that infection level has more influence on effectiveness of deep covering than on effectiveness of nondirting. It is evident in Fig. 1-B that: a) deep covering was not particularly effective at low and intermediate infection levels; b) the effectiveness of nondirting is influenced little by infection level; and c) deep covering was never quite as effective as nondirting, although the benefits obtained at higher infection levels were enough to justify making the deep, nondirting combination a standard recommendation.

The infection and yield data for all 5 years combined are shown in Fig. 1-C. There the high degree of inverse correlation between infection and yield is strikingly apparent. Note the low level of infection and high yield associated with the deep-covering, nondirting combination.

Still more evidence in favor of deep covering and nondirting is found in data obtained by scratching nuts out of the soil following digging of the peanuts in the plots receiving the different treatments in 1956 and 1957 (8). Careful observations led to the conclusion that the great majority of these nuts were left in the soil because the attaching pegs were rotted off by *S. rolfsii*. Even in the deep-covered, nondirted plots, almost 400 pounds per acre of nuts were lost in the soil as a result of peg-rot phase of stem rot. But the poorest treatment resulted in 3 times as much nut loss, indicating that the deep-covering, nondirting treatment is a partial answer to the peg-rot problem.

Whether these measures can be effective with other

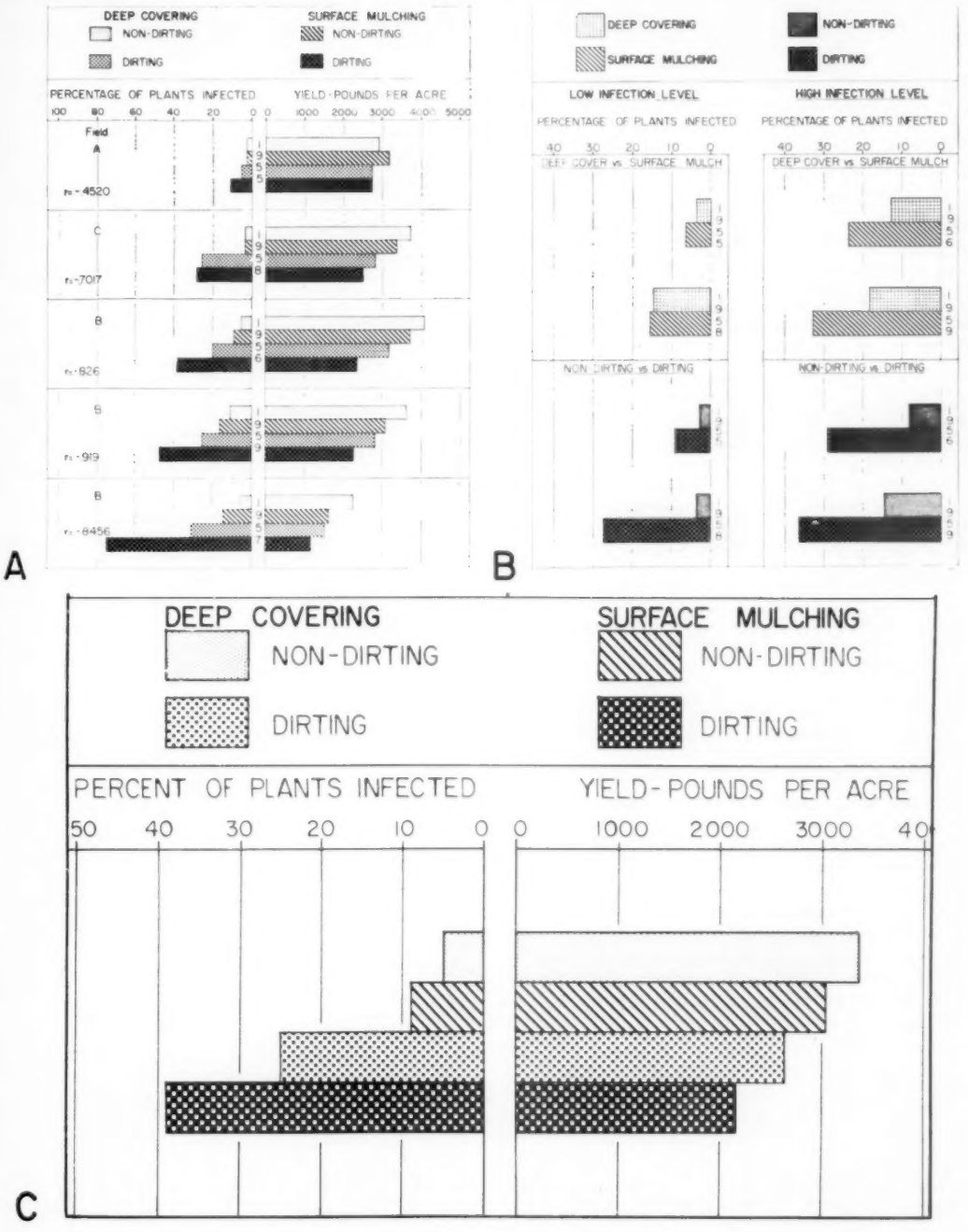

Fig. 1.—A) Relation of yields of Virginia Bunch 46-2 peanuts given 4 different treatments to *S. rolfsii* infection for a 5-year period in Virginia, and coefficients of correlation (r) for yield and infection. (Results in order of infection level, not in chronological order.) **B)** Influence of level of *S. rolfsii* infection on response of Virginia Bunch 46-2 peanuts to cultural treatments in Virginia. **C)** Relation of 5-year average yields of Virginia Bunch 46-2 peanuts given 4 different treatments to *S. rolfsii* infection.

crops remains to be seen. Field crop plants such as alfalfa and lespedeza, which are grown without cultivation, are occasionally severely attacked by *S. rolfsii* in Virginia. Obviously, only deep covering is applicable to these crops, and the writer feels that in such instances beneficial results would come from removal of much organic matter from the infection zone by deep covering. Deep covering might not be feasible in areas where it might increase wind erosion greatly.

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CONTROL OF SCLEROTIUM ROLFSII WITH CHEMICALS

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Numerous attempts to control *Sclerotium rolfsii* Sacc. by chemical means have been made for years, but the experiments usually met with mediocre results or complete failure. Standard fungicides failed to meet the requirements, and none of the early reports gave any real solution to the problem of controlling *S. rolfsii* with chemicals. This paper cannot review all the early attempts at chemical control, but attempts to clarify the picture with a brief summary of some of the work with fungicides and nitrogenous fertilizers.

Rolfs, in 1893 (31), thought that copper fungicides in the soil might reduce losses from *S. rolfsii*. Since then, numerous workers have reported varying degrees of control with copper fungicides, sulfur, and lime, but none of these treatments was sufficiently lasting for wide use. In special cases soil fumigation has been used to control *S. rolfsii*. Not until pentachloronitrobenzene (PCNB or Terraclor) was found to have fungicidal effects on *S. rolfsii* was progress made on a practical control of *S. rolfsii* with a fungicide under field conditions. Some reports have indicated that nitrogenous fertilizers might help reduce losses from *S. rolfsii* with some crops.

Nitrogenous fertilizers.—The nutrition of the host plant has been known for a long time to play an important part in its susceptibility to certain diseases. Indications that this might be true have been reported for *S. rolfsii* on certain crops. Leach and Davey, in 1935 (24), reported that applying anhydrous ammonia or ammonium sulfate in the irrigation water would suppress a root rot of sugar beets caused by *S. rolfsii*. They later reported (25) that fertilizing sugar beets with ammonium sulfate, anhydrous ammonia, calcium nitrate, or cyanamide at 50–200 lb nitrogen per acre was helpful for the control of *S. rolfsii*. In 1938 Poole (29) indicated that beneficial results may occur when some inorganic elements are added to fertilizer and from applications of sulfur and lime. In 1947 McClellan (26) reported that heavy applications of ammonium nitrate or uramon as side-dressings to annual larkspur would reduce losses from *S. rolfsii*. Chowdhury, in 1946 (4) and again in 1948 (5), reported that the death rate of *Piper betle* L. could be reduced by adding nitrogenous fertilizers at 50–200 lb nitrogen per acre, and that the quantity of nitrogen was more important than the type of nitrogen. Hudgins (22) reported that the nitrogen supply will influence the susceptibility of peanuts to southern blight. B. C. Langley (correspondence), however, failed to find any correlation between the severity of southern blight of peanuts and different

rates and ratios of nitrogen, phosphorous, and potash under field conditions. Johnson (23) suggested that ditions more favorable for antagonistic microflora, thereby controlling *S. rolfsii* indirectly.

Interest in nitrogenous fertilizers for the control of southern blight was renewed when Watkins et al (32) reported that side-dressing tomatoes with 150–200 lb calcium nitrate per acre would reduce the incidence of southern blight and delay its appearance. Young (34, 35) and Mohr and Watkins (28) presented further data that fertilizing with calcium nitrate will help delay the appearance of southern blight of tomatoes. Young (37), however, reported that, in a single test in 1959, calcium nitrate failed to protect tomatoes adequately under epiphytotic conditions.

Soil fumigants.—Many tests over the past several years have indicated that *S. rolfsii* can be controlled by soil fumigation. Soil fumigation tests with chloropicrin (1, 7, 14, 27) and methyl bromide (14) and formaldehyde (7) as a soil drench have been recommended as control measures. A comparatively new soil fumigant, chlorobromopropene, has reduced losses from *S. rolfsii* in tomatoes (1, 34, 38). Haasis (17) reported that treating the soil with chlorobromopropene reduced losses from *S. rolfsii* in Dutch iris, when the bulbs had been treated with formaldehyde, but that other organisms would cause trouble if untreated bulbs were planted in the treated soil.

In contrast to soil fumigation with chloropicrin, soil treatment with D-D (dichloropropene and dichloropropane) has been shown by Gould (16) and Rankin and Good (30) to increase the severity of *S. rolfsii*. Soil fumigation has limited possibilities for the control of *S. rolfsii* except in special cases such as gardens, greenhouses, or for specialized crops. It is of doubtful value in large-scale field operations for medium or low income crops.

Herbicides.—There are reports that some herbicides may have fungicidal properties. In 1956 Chappell and Miller (3) reported that peanuts treated with dinoseb (4,6-dinitro-*o*-secondary butylphenol) for weed control had fewer plants dead from *S. rolfsii* than the unsprayed rows. Then Garren (11, 12) reported that not only were fewer plants infected following applications of dinoseb, but that increases in yields were obtained in the 1956, 1957, and 1958 tests. The increases were statistically significant in 1956 and 1958, but not in 1957. These reports indicate that applications of dinoseb may help in the over-all program of controlling southern blight of peanuts.

Other chemicals.—A wide variety of chemicals has been used in the past few years in attempts at practical control of southern blight. Neither time nor space permits listing the numerous chemicals used. Some, such as dehydroacetic acid, have shown promise in the laboratory but failed to show effective control under field conditions (1, 18). A fungicide for the practical control of *S. rolfsii* must be stable in the upper 1–2 in. of soil. It must not be adversely affected by extreme fluctuations of moisture, temperature, etc., of the soil. Such a fungicide was discovered when pentachloronitrobenzene (PCNB or Terraclor) was found to have fungicidal activity against *S. rolfsii*. Hartzfeld (21) described the fungicide, formulations, possible uses, and some of its limitations. PCNB has been found to be useful on a number of crops for the control of *S. rolfsii*. It has been used on some ornamentals, vegetables, and peanuts with varying success.

Ornamentals.—The first report that PCNB was a possible fungicide for *S. rolfsii* on ornamentals was made by Gould in 1954 (15, 16). He reported that 200–300 lb of active material per acre would control *S. rolfsii* on Dutch iris. Since then his tests have repeatedly shown that PCNB will effectively control *S. rolfsii* on Dutch iris (correspondence). PCNB has been applied broadcast, in the furrow, and as a bulb dip. If the bulb dip is used, a 1% seed coat sticker is added to the PCNB suspension, 5–7½% by weight of a 75% wettable powder. In silt loams and peat soils the dip plus 150 lb active material per acre in the furrow is recommended, or 200 lb/A. in the furrow if the dip is not used. On sandy soils the rate of application is reduced by 50 lb/A. Aycock (2) reported that PCNB is an effective fungicide for *S. rolfsii* on Dutch iris in North Carolina. P. E. Nelson indicated (correspondence) in preliminary tests in New York that PCNB is promising as a fungicide for *S. rolfsii* on delphinium. PCNB was somewhat phytotoxic, at 200 lb active per acre, but not at lower rates. He further stated that additional tests will be necessary before definite recommendations can be made.

Vegetables.—Published information on PCNB for the control of *S. rolfsii* on vegetables is very meager. Results were promising in Texas (32, 35, 36, 37) when PCNB was applied to the base of tomato plants either as a drench or as a dust. At present no definite recommendations on the use of PCNB on tomatoes have been made in Texas. Diener (9) in Alabama, Dempsey and Tereshkovich (8) in Georgia, and W. E. Cooper (correspondence) in North Carolina, have recommended using PCNB in the transplant water to control *S. rolfsii* on pimiento peppers. The recommendations range from 2–4 lb of 75% wettable powder in 100 gal. water with one-third to one-half pint applied to the base of the transplant. Diener (9) also recommended the same treatment for tomato transplants.

Results in Texas (36, 37) and Georgia (H. W. Rankin, correspondence) indicate that though PCNB may reduce and delay the development of southern blight on tomatoes, it can be highly phytotoxic to tomatoes under some conditions. The safety range between effective control and phytotoxicity appears very narrow. Additional tests will have to be made before PCNB can be safely recommended for the control of *S. rolfsii* on young tomatoes in some areas.

Peanuts.—The first report that southern blight of peanuts could be controlled with PCNB was made by Cooper in 1956 (6). He reported that 6 and 12 lb active PCNB per acre would reduce the stem rot phase

of southern blight and also increase yields. Since then other workers (12, 18, 19, 20 and correspondence with D. F. Cohoon and W. E. Cooper) have reported that Terraclor may be used to reduce losses from *S. rolfsii*.

Following results obtained in 1957 and 1958 (18, 19, 20), testing PCNB for the control of *S. rolfsii* on peanuts was expanded in Texas in 1959 to include replicated and observational tests in fields under commercial conditions. Most of the large field tests were on irrigated peanuts. All the work was done with commercial equipment. In 1958, PCNB was applied as a 10% dust with Howry Berg Soil Chem applicators, and in 1959 a Gustafson Soil Chem mixer was used. All applications in the commercial field tests were made just before or just after planting, so that the peanuts would be on slightly raised beds and in treated soil. A runner planter was used when the PCNB was applied before planting. The fields were relatively free of weeds, requiring little or no dirting during cultivation. There was very little undecomposed surface organic matter at time of planting. Over 1000 acres of irrigated peanuts were treated in 1959 in Frio County, in South Texas.

The data from these tests demonstrated that PCNB applied at planting time will not only increase the yield of nuts but also increase the grade. Even under conditions where *S. rolfsii* was of minor importance, PCNB gave, in a few tests, significant increases in yields even when applied at a rate as low as 5 lb/A. In tests where *S. rolfsii* was of major importance, PCNB increased yields as much as 904 lb/A., with an increase of 12% in sound mature kernels.

In the 1959 replicated tests at Yoakum, Texas, PCNB was applied: 1) in a spray to the crown of the peanut plants at cultivation time; 2) as a dust in a 12-in. band around the plants just before cultivation; or 3) as a dust at planting time. When applied at planting time, a band 10–12 in. wide was distributed over the row and mixed with the top 1–2 in. of soil with a Howry Berg rotary mixer. Cultivation was by conventional means so that some soil was pushed around the crown of the peanut plants. Some supplemental irrigation water was applied in some tests, though not in sufficient quantities to supply all the needs of the crop. These data are summarized in Tables 1 and 2. The level of infection from southern blight was relatively light. Nevertheless, PCNB gave significant increases in yield. PCNB was more effective when applied in split applications than when applied in a single application as a dust or as a spray. Although the data in Table 1 indicate the deep covering of the organic matter during land preparation will help control southern blight, tests by G. M. Watkins and B. C. Langley (unpublished data) and unpublished results of the author have failed to show any differences in yields of nuts following the suggested deep burial of the surface organic matter.

PCNB—Gypsum.—Calcium has long been recognized as an important nutrient element in peanut production. To meet this requirement, calcium in the form of calcium sulfate (gypsum) has been applied during the

Table 1.—Effect of application method on the control of southern blight of peanuts with PCNB.

Method of application	Av. lb of nuts per acre		
	S.M.[a]	D.C.[b]	Av.
Spray at each cultivation[c]	1685	1844	1764
Dust at each cultivation[c]	1666	1774	1720
Dust at planting time[d]	1508	1800	1654
None	1450	1731	1590
Average	1577	1787	

[a] Surface mulching of organic matter during land preparation.
[b] Deep covering of surface organic matter during land preparation.
[c] PCNB was applied at 6, 9, 12, and 15 lb active per acre with one-third of total applied at each of 3 cultivations.
[d] PCNB was applied at 5, 10, and 15 lb active per acre at planting time. LSD @5% for method of application, 94. LSD @ 5% for method of land preparation, 123.

Table 2.—Effect of application method on the control of southern blight of peanuts with PCNB.

Method of application[a]	Av. lb of nuts produced per acre
Spray at each of three cultivations (av. all rates)	1450
Dust at planting time (av. all rates)	1324
Spray at second cultivation (av. all rates)	1320
Spray at first cultivation (av. all rates)	1274
Dust at third cultivation (av. all rates)	1257
Spray at third cultivation (av. all rates)	1231
Check (none)	1052
LSD @ 5%	116

[a] PCNB was applied at 6, 9, and 12 lb active per acre with each method. With the first method, one-third of the above rates was applied at each cultivation. In all other methods the total amount was applied at the time indicated.

Table 3.—Effect of PCNB and PCNB plus gypsum on the control of southern blight of peanuts.

Test	PCNB lb/A.	Yield in pounds of nuts per acre			LSD @ 5%
		PCNB	PCNB + Gypsum	Check	
A. L. Harrison '58[a]	9	411	454	399	n.s.
A. L. Harrison '58[a]	18	405	417	399	n.s.
A. L. Harrison '58[a,b]	9	1646	1567	1404	232
A. L. Harrison '58[a,b]	18	1815	1428	1404	232
A. L. Harrison '58[c]	15	1233	1257	1210	
W. E. Cooper '56[d]	12	1678	1254		
D. F. Cohoon '59[e]	25	1597	1366	969	156
K. H. Garren '57[f]		2775[g]	2585[g]	2636	[h]
K. H. Garren '58[f]		3012[g]	2974[g]	2866	n.s.

[a] These data from a test comparing PCNB on irrigated and unirrigated peanuts.
[b] Irrigated peanuts.
[c] Data from a commercial field in Texas.
[d] Unpublished data from North Carolina.
[e] Unpublished data from South Carolina.
[f] PCNB, 12 lb active per acre.
[g] 15 lb active PCNB + gypsum per acre.
[h] PCNB significant @ 5% level over PCNB + gypsum.
[i] Garren (10).

growing season (33). Therefore, some tests were conducted in different states to see if a PCNB + gypsum mixture could be used to control *S. rolfsii* and also supply the calcium needs. Table 3 presents data from such tests in North Carolina, Virginia, South Carolina, and Texas. The PCNB+gypsum mixture was applied at pegging time at about 400 lb/A. These data suggest that PCNB is more effective in controlling southern blight when used alone than when used in combination with gypsum, and definitely points to the need of additional work in an attempt to solve some of the problems arising from the use of PCNB + gypsum mixture.

Discussion.—PCNB has been definitely shown to be an effective fungicide for the control of *S. rolfsii*. Even so, the economics of its use is a question of paramount importance. Only where the relatively high cost of PCNB can be absorbed can it be used economically. The unpredictable nature of *S. rolfsii* (13) and uncertainty in the weather add to the problem of deciding whether PCNB can be used profitably. Coupled with these uncertainties, there is the possibility of having a disease complex. Such a situation occurred in two tests on Spanish peanuts in Texas in 1959. Application of PCNB failed to increase the production of nuts. A close examination revealed that several species of nematodes and fungi were involved. *S. rolfsii* apparently played only a minor role in the disease situation. These observations emphasize the importance of having a broad disease control program so that all "root rots" of the peanut will be controlled.

Many factors determine the effectiveness of PCNB for the control of *S. rolfsii* on peanuts. These factors probably play the same role with other crops. The PCNB must be mixed thoroughly in the top 1–2 in. of soil in the pegging zone of the peanut plant if protection is to be maximum. Any cultural practices that will bury the treated soil with untreated soil will decrease the effectiveness of the PCNB. Burying fresh organic matter around the crown of the peanut plants from weeds or peanut leaves will also reduce the effectiveness of PCNB. A program that has been highly successful for the control of *S. rolfsii* on Spanish peanuts in the irrigated areas of South Texas has been to plant on raised beds, apply and mix thoroughly 10–15 lb of PCNB per acre in a 10–12 in. band over the row at planting time, cultivate for weed control with beet knives and rotary hoes, and dust with sulfur for the control of the Cercospora leaf spots and rust. Growers who consistently followed these practices in 1959 frequently exceeded 3000 pounds of nuts per acre.

Although progress has been made on the chemical control of *S. rolfsii*, additional research is urgently needed to find solutions to some of the inconsistencies obtained with PCNB in different locations.

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PHYTOPATHOLOGICAL NOTES

THE TOXICITY OF SOME CHLORINATED PHENOLS AND ARYLOXYALKANECARBOXYLIC ACIDS TO VERTICILLIUM ALBO-ATRUM

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It has been reported that aryloxyalkanecarboxylic acids, such as 2,4-dichlorophenoxyacetic acid (2,4-D), are not particularly toxic to *Verticillium albo-atrum* Reinke & Berth. (4). Certain laboratory tests made in conjunction with field trials (5), however, indicated that some of these compounds were quite toxic to the fungus. A study was made to determine: 1) the relation of the structure of some chlorinated phenols and aryloxyalkanecarboxylic acids to toxicity, 2) the effect of pH and media composition on toxicity, and 3) whether *V. albo-atrum* could metabolize these acids.

The toxicity tests were made in a synthetic liquid medium (8). The chemical to be tested was dissolved in 50% acetone. The acids were neutralized by adding a stoichiometric amount of sodium bicarbonate to the acetone solution. One ml of the acetone solution was added to each flask of autoclaved medium. The flasks were seeded with a mycelial suspension of the fungus and mycelial mats harvested at regular intervals.

Experiments were made with several isolates of *V. albo-atrum* to determine the approximate concentration of compound that would inhibit mycelial mat weight about 50% 14–18 days after seeding (8). The compounds differed quite widely in toxicity despite variations among the isolates and experiments. Table 1 summarizes the results. It was found that: 1) the phenol was more toxic than the corresponding aryloxyalkanecarboxylic acid, 2) adding chlorine atoms to the ring increased the toxicity of both phenols and acids, and 3) adding carbon atoms in the side chain increased the toxicity of the acids.

Some of the acids were quite toxic to the fungus, and some compounds were more toxic than known metabolic poisons such as 2,4-dinitrophenol. Generally, the compounds were more toxic to the fungus than reported (4). Earlier results were obtained with the fungus grown on potato-dextrose agar at pH 5.0–5.6. Other workers reported that 2,4-D was more toxic to some fungi when the pH of the medium was low (3).

Several experiments were made to determine the effect of the pH of the medium on toxicity. The *V. albo-atrum* in these experiments was inhibited when the initial pH was about 3.2 (7). At pH 3.5–3.7, there was a slight lag period but the fungus grew well. The toxicity of the chlorinated phenols or acids was enhanced when the pH of the medium was low. In one experiment 2,4-D ($10^{-3}M$) was added to media adjusted to pH 3.5, 4.5, and 6.5, and the growth was compared to that in control media of the same pH. Inhibition was almost complete at pH 3.5, 35% at pH 4.5, and little if any at pH 6.5. Results were comparable with $6\times10^{-5}M$ 2,4-dichlorophenol. This increase in toxicity at low pH and increase in toxicity with addition of chlorine atoms suggest that these

Table 1.—The concentration of various chemicals required to inhibit mycelial mat production of *Verticillium albo-atrum* about 50% in a synthetic liquid medium (pH 4.5). Mat weights determined 14–18 days after seeding the liquid medium.

Chemical	*M*
Phenols	
2,4-dichlorophenol	6×10^{-5}
2,4,5-trichlorophenol	10^{-5}
2,4,6,-trichlorophenol	10^{-5}
2,3,4,5,6-pentachlorophenol	2×10^{-6}
naphthol	10^{-4}
Acids	
Phenylacetic acid	$>10^{-3}$
Phenoxyacetic acid	$>10^{-3}$
Phenoxypropionic acid	$>10^{-3}$
p-chlorophenoxyacetic acid	$>10^{-3}$
2,4-dichlorophenoxyacetic acid	10^{-3}
2,4,5-trichlorophenoxyacetic acid	5×10^{-4}
2,4,6-trichlorophenoxyacetic acid	5×10^{-4}
2,3,4,5,6-pentachlorophenoxyacetic acid	6×10^{-6}
2(2,4-dichlorophenoxy)-propionic acid	5×10^{-4}
4(2,4-dichlorophenoxy)-butyric acid	5×10^{-5}

compounds may exert their effect after penetrating the cell (6). Other experiments have shown that in media at an initial pH of 5, 2,4-D was more toxic to the fungus in the synthetic medium than in potato-dextrose broth.

Experiments were conducted to determine if the fungus could metabolize phenoxyacetic acid and 2,4-D. In some experiments sucrose was omitted from the medium (pH 4.5 and 6.5) and nontoxic levels of the compounds were added as the only carbon source. The fungus did not grow in such media. In other experiments phenoxyacetic acid or 2,4-D was added to the medium containing sucrose or glucose. Periodic harvests were made by blending medium and mycelium and boiling this material with 2 volumes of ethanol. The cooled mixture was filtered; the filtrate was concentrated, acidified to pH2, and extracted 3 times with diethyl ether; and the extract was reduced to a small volume and applied to Whatman No. 1 chromatography paper. The chromatograms were irrigated with isopropanol-ammonia-water (10:1:1, v/v) and sprayed with aniline xylose reagent (1). Neither compound appeared to be metabolized by the fungus.

It has been shown that foliar applications of 2,4-D can decrease symptoms of Verticillium wilt of potatoes (5,9). Studies on growth regulators of the 2,4-D type for the control of other diseases have shown there is insufficient material in the plant to act as a fungicide. It is possible that the plant may metabolize these compounds to more toxic compounds; however, no fungicidal materials have been demonstrated in the treated plants. These facts have led to the theory that plant growth regulators exert their effect by altering the metabolism of the host (2). The present experiments indicate that any fungicidal effect of these compounds might also be modified by the pH of the tissue and the presence of other compounds in the plant. The effect of pH and medium composition on toxicity should also be considered when bioassays for active materials are made on plants treated with growth regulators.—Agricultural Chemistry Department, University of Idaho, Moscow.

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CERCOSPORA LEAFSPOT OF STATICE

Curtis R. Jackson

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Commercial statice production in Florida is presently confined to a small acreage mostly planted to 2 species, *Limonium sinuatum* (L.) Mill and *L. bonduellii* (Lest.) Kuntze. Both species are subject to a leafspotting disease caused by *Cercospora insulana* Sacc. The disease, reported by Chupp (1), has been noted frequently in several commercial plantings. Losses have usually been slight since the disease commonly appears after flowers have developed (January–March), but heavy leaf infection frequently results in defoliation and the death of immature panicles. This report presents information on the morphology, pathogenicity, and growth *in vitro* of the pathogen.

Lesions on leaves and foliar wings of the panicle branches are initially dull red to orange, 0.5–1 mm in diameter, later becoming centrally membranous, tan, up to 15 mm in diameter, and surrounded by narrow reddish-brown or orange margins (Fig. 1). Sporulation is equally abundant on both surfaces. Lesions become confluent and spread during warm, wet weather.

Pathogen morphology.—Samples of conidia and conidiophores were taken from naturally occurring lesions on several leaves from 1 collection (Fig. 2). The samples were mounted in water, and measurements were made of conidia and conidiophores chosen at random. The measurements of 50 conidia and 25 conidiophores are given in terms of ranges and crude modes. The latter are of more interest than means since they indicate the approximate values of length, width, and septa numbers most frequently encountered in the samples. The modes were calculated from the formula crude mode = 3 median − 2 mean, and all modes except that of conidiophore width were smaller than corresponding means. The frequency distribution diagram for conidiophore width was skewed toward large values and the other diagrams were skewed oppositely. Conidia were 58–325 μ (mode 90 μ) long, 2.6–5.8μ (mode 3 μ) wide, with 2–24 (mode 8) septa. Sixty-two per cent of the conidia had truncate bases; the remainder were bluntly rounded. Conidiophores were 39–110 μ (mode 57 μ) long and 3.2–5.6 μ (mode 5 μ) wide. These measurements and other morphological features conform closely to Chupp's (1) description.

Pathogenicity.—Isolations from leaf lesions were grown in pure culture on potato-dextrose agar, and a

Fig. 1.—Cercospora leafspot symptoms on leaves of *Limonium sinuatum* 4 weeks after inoculation.

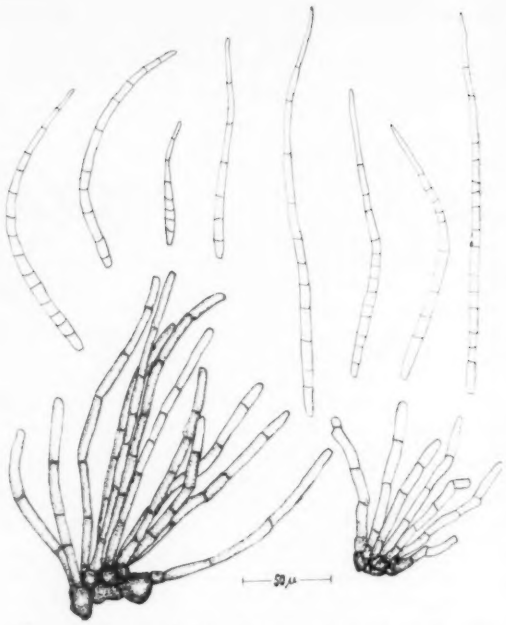

Fig. 2.—Camera lucida drawings of conidia and conidiophores of *Cercospora insulana* from host.

water suspension of mycelial fragments from these cultures was used to inoculate leaves of 10-week-old plants. Besides *L. bonduellii* and the white, rose, and lavender color-variants of *L. sinuatum*, plants of *Plumbago capensis* Thunb. were used. *P. capensis*, an ornamental widely grown in Florida, and statice are both members of the Plumbaginaceae. The mycelial suspension was atomized on the leaves, and, after 2 days in a moisture chamber, the plants were removed to a greenhouse bench. Leaf symptoms were noted on both species of statice within 13 days of inoculation, and the pathogen was reisolated from lesions. Leaves of *P. capensis* did not become infected.

In a second inoculation experiment, a water suspension of conidia from the surfaces of numerous naturally occurring lesions was atomized on potted mature *L. sinuatum* plants. The moist plants were then sealed in polyethylene bags. Three inoculated replications and 1 bagged control plant were placed in each of 5 lighted constant-temperature chambers at 12, 16, 20, 24 and 28°C. After 5 days the plants were removed from the bags and placed together on a greenhouse bench at 20–32°C. Disease ratings were made 16 days after inoculation.

Discrete lesions developed on some leaves of all inoculated plants, and control plants remained uninfected. The mean numbers of lesions per leaf from the various temperatures were: 16°C–21, 12°C–19, 24°C–14, 20°C–12, and 28°C–6. About the same number of leaves were infected at all temperatures.

Growth In vitro.—The cultural and analytical methods used to determine the optimum temperature for mycelial growth were those described by Jackson (2), except that the growth period was reduced to 9 days, replications per temperature were 8, and treatments were added at 12 and 36°C.

Mycelial growth was maximum at 28°C and significantly different at the 5% level from growth at any other temperature. Growth decreased in the order 20, 24, 16, 12, 32, 36°C.—Gulf Coast Experiment Station, University of Florida, Bradenton.

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LOCAL INFECTION OF RICE FLORETS BY THE RICE KERNEL SMUT ORGANISM, TILLETIA HORRIDA

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The mode of infection of the rice kernel smut pathogen, *Tilletia horrida* Tak. (= *Neovossia barclayana* Brefeld), is incompletely understood. Seedling infec-

tion has been indicated by Anderson (1), Reyes (4), and Tullis and Johnson (5). Embryo infection was indicated by Walker (6). Local infection of florets, recently demonstrated by Chowdury (2), is supported by observation, i.e., usually no more than 3–4 kernels are infected in each panicle, and distribution is frequently random throughout the panicle.

Preliminary tests in the greenhouse at Fayetteville support the work of Chowdury. A low but significant amount of infection was obtained by subjecting CI 9187 rice panicles at anthesis to a shower of secondary sporidia from an inverted plate of germinating chlamydospores. The plate and panicles were enclosed in a large polyethylene bag for the period of inoculation (5 days). Eight of the ten panicles inoculated contained 1–2 infected kernels, whereas none of the 300 uninoculated panicles was infected. The plants were grown from hot-water-treated seed (3) that had been hand-picked with the aid of a transmitted light to remove partially smutted kernels. The soil was not sterilized but was taken from the University Farm at Fayetteville, which is over 200 miles from the rice-growing area of the state and has never had rice grown on it. These precautions, together with the lack of infection in the controlled plants, indicate that these infections were not the result of seedling or embryo infection but local infection of the rice florets. —Department of Plant Pathology, University of Arkansas, Fayetteville.

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APHANOMYCES ROOT ROT OF PEAS AS AFFECTED BY ORGANIC AND MINERAL SOIL AMENDMENTS

C. B. Davey and G. C. Papavizas

Relatively little information has been published on the effect of plant materials in soil (organic amendments) on development of the root rot of peas caused by *Aphanomyces euteiches* Drechs. Johnson (4) showed that a green manure precrop of rye reduced disease severity somewhat whereas barnyard manure was ineffective. Lyda (5) reported that several organic amendments added to sterilized soil inoculated with *A. cochlioides* Drechs., a root rot pathogen of sugar beets, did not reduce the disease, whereas most of the same amendments added to inoculated unsterilized soil gave some, though variable, control. He concluded that the effect of the amendments was indirect and was regulated by their effect on the whole soil flora.

Several workers (2, 3, 4, 6) reported that nitrogen reduced damage caused by Aphanomyces root rot of peas. Geach (2) found that sodium was detrimental to the causal organism *in vitro* and reduced disease severity when added to infested soil. He applied sodium as the nitrate salt, however, making it difficult to differentiate between the effects of sodium and nitrogen. Smith and Walker (7) concluded that a high osmotic value of the soil solution was responsible for disease reduction rather than the presence or the absence of specific nutrients. Wade (8), however, obtained considerable disease reduction by low applications of potassium to a soil with a low salt content.

The present investigation was undertaken to determine the effects of several organic amendments and mineral salts of nitrogen and sodium on the Aphanomyces root rot of Alaska peas.

Greenhouse ground beds were filled with unsterilized Elsinboro sandy loam. The soil was infested with a mixture of 3 isolates of *A. euteiches* and repeatedly cropped to peas until infestation was heavy. The beds were then divided into 36 plots (4 x 4 ft) separated by buffer strips 1 ft wide. Twelve treatments were replicated 3 times in a randomized block design. Ground amendments, mature or nearly mature, were added to the soil at about 0.75% on a dry-weight basis of both soil and amendment 4 weeks before the peas were planted. Nitrogen, either as NH_4NO_3 or $NaNO_3$, was added to the soil at 300 lb N/A. (broadcast application) 2 days after planting. Sodium was applied as $NaNO_3$ or Na_2SO_4 at 490 lb Na/A. This rate was determined by the amount of sodium carried by the $NaNO_3$. Corn and oat amendments were added alone or in combination with NH_4NO_3. Peas, rye, soybeans, and sphagnum were incorporated singly with the soil. To ensure infection, soil moisture was increased to near saturation for several days when the seedlings were 4–6 in. tall.

Plants were carefully lifted and their disease indices determined when they were 4 weeks old. One hundred plants from the central portion of each plot were individually rated on an infection scale of 0 (no symptoms) to 4 (severe symptoms). A disease index of 0 to 100 was calculated from the individual ratings by multiplying the mean by 25. One week after the first crop had been removed from the soil a second planting was made and the same procedures of watering and disease rating were followed.

In both plantings the salts reduced disease severity effectively, with nitrogen being somewhat superior to sodium (Table 1). The oat amendment was consistently the most effective treatment in reducing disease severity. Sphagnum was responsible for a slight increase in the disease index above that obtained in peas planted in the control soil. In the second plant-

Table 1.—Aphanomyces root rot of Alaska peas as affected by organic soil amendments and mineral salts in first of two plantings.

Soil treatment	Disease index	Per cent stand
Oats	18 a[a]	90 ab
NH_4NO_3	23 ab	76 d
Oats + NH_4NO_3	29 ab	84 bc
Corn + NH_4NO_3	33 ab	83 c
$NaNO_3$	38 ab	35 c
Na_2SO_4	41 abc	76 d
Corn	43 abc	91 a
Peas	56 abcd	87 abc
Soybeans	61 abcd	89 abc
Rye	65 bcd	88 abc
None (control)	82 cd	85 abc
Sphagnum	85 d	89 abc

[a]Numbers followed by the same letter are not significantly different from each other at the 5% level of probability according to Duncan's multiple F test (1).

ing, oats and NH_4NO_3 were again the most effective treatments. In the first planting the salts significantly reduced seedling emergence and per cent stand. The oat or corn amendments partially ameliorated the salt damage when they were applied before NH_4NO_3. All organic amendments were responsible for pea stands slightly increased above those in the control soil. In the second planting emergence was fairly uniform regardless of treatment.

In a subsequent experiment the same organic amendments were added to the infested unsterilized soil when the amendment plants were immature. Sphagnum was again included for comparison. The soil was placed in No. 10 tin cans and soil moisture was controlled by daily weighing and watering. Soil moisture was maintained at 50% of moisture holding capacity (MHC) except during infection. When the plants were 4–6 in. tall, soil moisture was increased to 70% MHC for one week. All amendments except peas and sphagnum reduced disease severity substantially. The disease index exceeded 80 in the unamended control soil, and was less than 20 in soil containing corn, oats, rye, or soybeans.

The results suggest that certain mature or immature plant materials added to soil reduce Aphanomyces root rot of peas. They also reconfirm the value of nitrogen in suppressing this root rot. The superiority of nitrogen over sodium was consistent though lacking statistical significance at the 5% level.—Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

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RED CLOVER: A NEW HOST FOR POTATO VIRUS X

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It was recently reported that several viruses commonly found in red clover (*Trifolium pratense* L.) in Minnesota produced local lesions when applied to leaves of *Gomphrena globosa* L. (1). These local lesions were similar to those produced by inoculating *G. globosa* with sap from potatoes infected with virus X. It seemed possible, therefore, that red clover might be a host for potato virus X. At the time this possibility was being examined, workers at the University of Wisconsin were investigating crimson clover (*T. incarnatum* L.) as a host for the virus (2).

Virus-free plants from a clone of Wegener red clover were mechanically inoculated with crude sap from healthy or virus X (ringspot strain)-infected Kennebec, Lasota, and Triumph potatoes. The red clover had previously been cut back, and the first leaves to appear were inoculated after they had been dusted with 500 mesh Carborundum. Immediately after inoculation, the leaves were rinsed with tap water and the plants placed in a greenhouse at 75°F. Daylight was supplemented with artificial light, and the experiments were done in March and April, 1960.

Six weeks after inoculation, each of 60 red clover plants inoculated with sap from diseased potatoes had symptoms consisting of a diffuse mottle and a slight veinal chlorosis. Each of the 60 red clover plants inoculated with sap from healthy potatoes was free of symptoms. When sap from infected red clover was applied to leaves of virus-free potatoes, local lesions formed and were followed by severe mottle and puckering of young leaves. On *G. globosa*, *Nicotiana rustica* L., and *N. glutinosa* L., the infective red clover sap produced symptoms typical of those caused by virus X. No symptoms were produced when potatoes, tobacco, or *G. globosa* were inoculated with sap from symptomless red clover.

Crude sap from the pigmented areas surrounding local lesions on *G. globosa*, which was infective on

potatoes and red clover, produced typical symptoms of infection with virus X on both.

These results indicate that potato virus X can infect red clover and that red clover may be a reservoir of inoculum for the virus. Insect transmission from red clover was not studied.

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THE PRESIDENT'S COLUMN

What is the status of our Vice-Presidency and what should it be in relation to the Office of President-Elect and the Presidency? Should there be a direct line of succession from the Vice-President to the President-Elect and if so do we need both?

In the early years of our Society, there was very real competition among the candidates nominated for President. Presumably the Vice-President was usually, if not always, among the nominees. However, less than one-third of the Vice-Presidents were elected immediately to the Presidency though eventually another third attained the Presidency. A new pattern in our voting habits became apparent during the fourth decade of our Society. By 1946 this pattern apparently had become firmly established: namely, the Vice-President always has proved to the strongest candidate and has been immediately elected President.

In the meantime the Council, without the benefit of hindsight, became increasingly concerned about the possibilities of electing a green president without experience in the Society affairs which were becoming more complex. And so in 1950, the new office of President-Elect was created to insure that the President would have served at least a prior year on the Council. As it has turned out, this was not essential; however, most Presidents-to-be have probably gained from this extra year on the Council.

As it now stands, in the light of 16 uninterrupted years, there is a very reasonable expectation that each Vice-President will be elected President-Elect and a year later assume the Presidency. Now here is the joker. In accord with our Constitution the Vice-President, in emergencies, must assume the responsibilities of the President and in the ultimate, the office. In such an went the Vice-President would by-pass the President-Elect. In our situation this is completely illogical. The President-Elect by training and seniority should take the helm in an emergency.

The answer is relatively simple. By constitutional change, one of several options should be enacted: (1) Abolish the office of President-Elect and specify that the Vice-President shall assume the office of President the following year. (2) abolish the office of Vice-President and specify that the President-Elect shall assume the duties normally accorded a Vice-President; (3) keep both offices as now, but specify that the President-Elect shall be first in line of responsibility and succession to the Presidency.

We should point out that there has been some interest in another approach. It is felt by some that our Society is now sufficiently large to elect several Vice-Presidents. They would share in the honors and responsibilities. Presumably, they could be elected on a divisional or geographical basis, on a subject matter basis, or at large. The last would seem preferable. With several Vice-Presidents, probably the President-Elect should be eliminated. However, at the present time is there a real need for more than one Vice-President?

Until very recently the Vice-President, as is common, has been essentially without specific duties. He is now, by custom, chairman of the Membership Committee. However, this chairmanship could be readily handled by others as in the past. The President-Elect has just been given extensive responsibilities, having been made Program Chairman. If necessary, these duties could be assigned to the Vice-President. In our opinion, events have made it clear that we do not need both a Vice-President and a President-Elect. Furthermore, in line with the wishes of a plurality of our members as shown by their voting records for the past 16 years, we favor the first option. That is, we should eliminate the more or less token election for President-Elect and vote for a Vice-President who will accede to the Presidency a year later.

S. E. A. McC.

NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

The annual oak wilt conference was held in Bedford, Pennsylvania, December 1 and 2, 1960. The conference was attended by pathologists, foresters, and U. S. Forest Service personnel from West Virginia, Pennsylvania, Maryland and Virginia. State and Station survey and research reports were given. A committee was formed to study the importance and value of oak and its diseases in these states. The committee will report at the 1961 meeting on proper balance of oak wilt research and its relation to other forest disease problems. Proceedings of the 1960 oak wilt conference will be available from the Division of Forest Disease Research, Northeastern Forest Experiment Station, Upper Darby, Pennsylvania.

Phytopathologist Charles J. Nusbaum, North Carolina State College, Raleigh, presented a public lecture at the college on November 30, 1960, entitled "Nematode Population Dynamics in Relation to Tobacco Culture." The lecture was sponsored by the local chapter of the Society of Sigma Xi, of which Dr. Nusbaum is immediate Past-President.

The University of North Carolina Press recently announced the publication of a book entitled "Nematology—Fundamentals and Recent Advances with Emphasis on Plant Parasiitc and Soil Forms" edited by J. N. Sasser and W. R. Jenkins. The book was compiled from a series of lectures and laboratory exercises presented during a Southern Regional Graduate session in Advanced Nematology held at North Carolina State College in 1959. This publication has 494 pages, 162 figures and 79 tables, and contains 47 articles by 22 eminent nematologists.

Pathologist W. B. Hewitt, University of California at Davis, visited the Plant Pathology Department, North Carolina State College, November 29-December 3. Dr. Hewitt presented two special seminars dealing with soil-borne viruses and nematodes as vectors of plant viruses.

The voters of North Dakota elected, on November 8, 1960, to change the name of their land grant institution from North Dakota Agriculture College to North Dakota State University. The Plant Pathology Department is in the College of Agriculture at the University.

Extension plant pathologist Furney A. Todd, North Carolina State College at Raleigh, visited West Germany, France, and Switzerland in late August and early September 1960, to study the tobacco downy mildew situation and advise on possible control measures. Dr. Todd reported that the disease, first noted in Europe in 1958, is now widespread and estimated that it destroyed fifty per cent of the tobacco crop in West Germany in 1960.

Plant pathologist George H. Hepting, Chief, Division of Forest Disease Research, Southeastern Forest Experiment Station, Asheville, North Carolina, recently spent four months leave from the U. S. Forest Service as special consultant to New Zealand Forest Products, Limited, a large company producing a wide variety of wood and paper products.

Pathologist David W. Rosberg has been named Head of the Department of Plant Physiology and Pathology at Texas A. & M. College, College Station, Texas. Dr. Rosberg replaced Dr. W. C. Hall who was appointed Dean of Graduate Studies in July 1960.

Wanted—Plant Disease Reporter 43, No. 7, 8, and 9 (July, August, and September, 1959) and Supplement No. 221 (September 1953). The Librarian, Morton Chemical Company, Route 14, South, Woodstock, Illinois desires copies of these issues.

Phytopathologist Graydon Kingsland was recently appointed Assistant Plant Pathologist in small grains research and Assistant Professor of Botany in the Department of Botany and Bacteriology at Clemson College. Dr. Kingsland was formerly with the United Fruit Company in Honduras.

Bacteriologist John H. Bond, Department of Botany and Bacteriology, Clemson College, Clemson, South Carolina, recently received a National Science Foundation Grant-in-aid of $6,400 covering a two year period. Professor Bond is making a study of soil microbial antagonisms and biological control of plant diseases.

Pathologist Wesley Witcher was recently appointed Associate Forest Pathologist and Associate Professor in the Department of Botany and Bacteriology at Clemson College. Dr. Witcher recently received his Ph.D. degree in plant pathology at North Carolina State College, Raleigh.

Pathologist Kiyowo Aoshima from the Government Forest Experiment Station, Tokyo, is spending the winter at the USDA Beltsville laboratory studying fungi related to forest tree diseases and wood deterioration.

Virologist Ellen Moorhead Ball, University of Nebraska, was a recent visitor at the Department of Plant Pathology, Cornell University. During her two week stay at Cornell she delivered a series of lectures on serology of plant viruses and consulted with members of the staff and students on serology.

Pathologist Kenneth D. Fisher recently received his Ph.D. degree from North Carolina State College and has accepted a position as Assistant Professor of Plant Pathology at South Dakota State College, Brookings. Dr. Fisher will conduct research on vegetable diseases.

Pathologist Thomas P. Pirone recently joined the staff of the Department of Plant Pathology at the Louisiana State University. Dr. Pirone received his Ph.D. degree from the University of Wisconsin in 1960 and will conduct research on vegetable and ornamental diseases in his new position.

The Department of Plant Pathology, Washington State University, has moved to its new quarters and facilities in the recently constructed plant science building on the campus at Pullman, Washington.

Pathologist A. G. Plakidas, recently retired from the Louisiana State University, Department of Plant Pathology, has returned to the University as Professor Emeritus of Plant Pathology.

Pathologist E. C. Blodgett is in Tel Aviv, on a one year leave of absence from the Washington Agricultural Experiment Station, Prosser, Washington, under a United Nations assignment. Dr. Blodgett will assist and advise the Israeli government in the establishment and operation of field plant quarantine stations. He will also train technicians in detection and identification of fruit tree viruses.

Two Agricultural Handbooks have been published by the Crops Research Division, U. S. Department of Agriculture. Free copies of these two publications, Handbok No. 165, "Index of Plant Diseases in the United States" and Handbook No. 174, "Potato Blight Epidemics throughout the World" may be obtained from Paul R. Miller, Epidemiology Investigations, Plant Industry Station, Beltsville, Maryland.

Pathologist D. E. Ellis, Chairman of the Department of Plant Pathology at North Carolina State College, Raleigh, has recently completed a survey of teaching and research programs in several wsetern states under funds provided by his institution.

Pathologist E. C. Pollard, Chairman of the Department of Physics at Yale University and Visiting Professor in Biophysics at the Pennsylvania Staet University presented a course entitled "Biophysics of Viruses" during the fall semester.